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ORIGINAL ARTICLE *Infertility*

Smoking in infertile women with polycystic ovary syndrome: baseline validation of self-report and effects on phenotype

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STUDY QUESTION: Do women with polycystic ovary syndrome (PCOS) seeking fertility treatment report smoking accurately and does participation in infertility treatment alter smoking?

SUMMARY ANSWER: Self-report of smoking in infertile women with PCOS is accurate (based on serum cotinine levels) and smoking is unlikely to change over time with infertility treatment.

WHAT IS KNOWN ALREADY: Women with PCOS have high rates of smoking and it is associated with worse insulin resistance and metabolic dysfunction.

STUDY DESIGN, SIZE, DURATION: Secondary study of smoking history from a large randomized controlled trial of infertility treatments in women with PCOS ($N = 626$) including a nested case–control study ($N = 148$) of serum cotinine levels within this cohort to validate self-report of smoking.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women with PCOS, age 18–40, seeking fertility who participated in a multi-center clinical trial testing first-line ovulation induction agents conducted at academic health centers in the USA.

MAIN RESULT(S) AND THE ROLE OF CHANCE: Overall, self-report of smoking in the nested case–control study agreed well with smoking status as determined by measure of serum cotinine levels, at 90% or better for each of the groups at baseline (98% of never smokers had cotinine levels <15 ng/ml compared with 90% of past smokers and 6% of current smokers). There were minor changes in smoking status as determined by serum cotinine levels over time, with the greatest change found in the smoking groups (past or current smokers). In the larger cohort, hirsutism scores at baseline were lower in the never smokers compared with past smokers. Total testosterone levels at baseline were also lower in the never smokers compared with current smokers. At end of study follow-up insulin levels and homeostatic index of insulin resistance increased in the current smokers ($P < 0.01$ for both) compared with baseline and with non-smokers. The chance for ovulation was not associated with smoking status, but live birth rates were increased (non-significantly) in never or past smokers.

LIMITATIONS, REASONS FOR CAUTION: The limitations include the selection bias involved in our nested case–control study, the possibility of misclassifying exposure to second hand smoke as smoking and our failure to capture self-reported changes in smoking status after enrollment in the trial.

WIDER IMPLICATIONS OF THE FINDINGS: Because self-report of smoking is accurate, further testing of smoking status is not necessary in women with PCOS. Because smoking status is unlikely to change during infertility treatment, extra attention should be focused on smoking cessation in current or recent smokers who seek or who are receiving infertility treatment.

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Key words: cigarette smoking / anovulation / hyperandrogenism / infertility / obesity

Introduction

Smoking is both common among women of reproductive age, and associated with poor reproductive outcomes including infertility and adverse pregnancy outcomes (Triche and Hossain, 2007). Obese women may initiate or continue to smoke as they believe that it helps curb further weight gain (Tomeo *et al.*, 1999). Because many women with polycystic ovary syndrome (PCOS) in the USA and developed world are obese, they may have higher prevalence rates of smoking than other groups of infertile women. We noted in our Pregnancy in Polycystic Ovary Syndrome Study (PPCOS I) of women recruited in the USA that nearly 40% of randomized subjects ($N = 626$) had a current or past smoking history (Legro *et al.*, 2006). Rates may be higher in women with PCOS in other countries. A large cohort study of women with PCOS ($N = 346$) from Germany reported that 28% were current smokers (Cupisti *et al.*, 2010) and one from Denmark ($N = 650$) reported that 40% were smokers (Glintborg *et al.*, 2012). Further, smoking in women with PCOS has been associated with worsening metabolic profiles, including adverse lipid profiles (Glintborg *et al.*, 2012) and increased insulin resistance (Cupisti *et al.*, 2010), in addition to the many serious long-term health effects of smoking (U.S. Department of Health and Human Services, 2010).

The accuracy of self-report of smoking has not been determined in an infertile female population. There are several reasons why self-report may not be accurate and in fact smoking may be underreported (Gorber *et al.*, 2009). First, because of the known association of smoking with subfertility in the population, women who are infertile or are pregnant may be stigmatized by smoking or admitting to smoking and thus underreport it to health care professionals (Shipton *et al.*, 2009; Dietz *et al.*, 2011). Secondly, many infertility programs in the USA, especially those reporting IVF outcome data to the Society of Assisted Reproductive Technology (SART) and the Centers for Disease Control (CDC), recommend or require smoking cessation before starting treatment, given associations with decreased pregnancy rates (Rosevear *et al.*, 1992) and increased pregnancy loss (Winter *et al.*, 2002). Thus there may be a disincentive to accurately report as it may prevent access to further infertility treatment. Finally, because infertility treatment can be highly stressful (Pasch *et al.*, 2012), ongoing treatment without success may induce relapse in those subjects who have given up or cut down on smoking prior to entering treatment.

We performed this study to determine the accuracy of self-report of smoking based on serum levels of cotinine (a metabolite of nicotine) and whether these cotinine levels changed over the course of infertility treatment. Further we wanted to explore the relationship between reproductive and metabolic risk factors with the degree of current smoking, as well as the response of these risk factors to infertility treatment.

Materials and Methods

We performed a nested case–control study, selecting subjects on their self-reported history of smoking from our PPCOS I Study. PPCOS I was a double-blind double dummy randomized controlled trial of clomiphene, metformin, or the combination of the two to treat anovulatory infertility for up to six treatment cycles in women with PCOS. We have previously reported the baseline characteristics and main outcomes of the study (Legro *et al.*, 2006, 2007). We utilized the sample repository and database from which to explore new hypotheses using these existing materials and data.

Study subjects

In the nested case–control study, we studied 148 subjects. Subject inclusion criteria were the following: a recorded smoking history at baseline, stored serum from two study visits (performed during the projected luteal phase of the initial treatment cycle and a later treatment cycle), and signed consent form from the original study allowing use of stored serum for future studies. This protocol was approved by the IRB at the Penn State College of Medicine. Based on the subjects' self-report at baseline, we identified three groups of subjects: those who were current smokers, those who reported a previous history of smoking and now were non-smokers (past smokers), and those with no history of smoking (never smokers). Because we wanted to verify the accuracy of their initial self-report over time, we elected to measure cotinine levels at two time points in the trial; one during the initial treatment cycle after the subjects had begun study medication (cycle 1) and the other 4 months later (cycle 5). For our secondary aim of analyzing reproductive and metabolic risk factors at baseline and follow-up, we utilized the entire PPCOS I cohort of 626 subjects who have previously been described (Legro *et al.*, 2006, 2007). The follow-up visit was collected at either the time of conception or the last completed visit if the subject did not conceive.

There were no data on accuracy of smoking self-report in this population; thus we were unable to perform a power analysis to determine sample size. Of the 626 original subjects, only 306 had both a cycle 1 and cycle 5 visit. We arbitrarily choose a sample size of half of this cohort or 150 subjects with 50 per smoking group. Thus for the never smoking group, we randomly selected 50 out of 192 subjects that had both cycle 1 and cycle 5 data. For the past smoker group, we randomly selected 50 out of 65 subjects that had both cycle 1 and cycle 5 data. For the current smoker group, we selected

49 out of 49 subjects that had both cycle 1 and cycle 5 data; however, 1 of these 49 subjects did not have enough sample to run the cotinine assay leaving 48 in this group.

Serum assays

Serum samples were thawed and prepared for cotinine quantification as follows: (i) an aliquot of 10 μl of serum was first spiked with 5 μl of 100 ng/ml of cotinine-D3, (ii) 30 μl of methanol was added and the sample vortexed and sat on ice for 10 min and (iii) after centrifugation at 16 000*g*, 4°C for 10 min, the supernatant of the sample was transferred to a high pressure liquid chromatography sample vial for liquid chromatography–mass spectrometry (LC-MS) analysis. Cotinine standards were purchased from Sigma-Aldrich (St Louis, MO, USA). LC-MS analysis was performed on Shimadzu UFLC 20ADXR LC system in-line with an ABSciex 5600 TripleTOF Q-TOF mass spectrometer at the mass spectrometry core facility at Penn State Hershey. The injection volume was 1 μl. Liquid chromatography was performed at 30°C using a Acquity UPLC BEH C18, 1.7 μm, 2.1 × 50 mm column (Waters, Milford, MA, USA) and a gradient system with the mobile phase consisting of solvent A:1 mM heptafluorobutyric acid and solvent B: methanol, at a flow rate of 0.4 ml/min. The gradient program used was: 2% B for 2 min; linear gradient to 95% B in 0.5 min; 95% B for 0.7 min; return to initial condition and equilibrate for 1 min before next sample injection.

Mass spectrometry experiments were performed using an AB-Sciex 5600 Triple-TOF mass spectrometer. The mass spectrometer was operated in positive product ion mode with the source temperature set at 450°C. The standard curve ranges for cotinine were 5–1000 ng/ml. Quantification analysis was performed by Multiquant software (AB-Sciex, CA). The calibration curve was constructed using a weighted (1/x) linear regression of peak area ratios of analytes over the deuterium-labeled internal standard versus the concentration of cotinine.

All samples for reproductive and metabolic hormones in the full cohort were analyzed in duplicate for each hormone and assays were performed by the University of Virginia Center for Research and Reproduction Ligand Assay and Analysis Core Laboratory as previously reported (Legro *et al.*, 2006). Testosterone (T) was measured by radioimmunoassay (RIA) (Coat-a-Count Kit; Diagnostic Products Corp., Los Angeles, CA, USA); assay sensitivity = 10 ng/dl; intra-assay CV = 5.0% and inter-assay CV = 8.2%. Sex hormone–binding globulin (SHBG) and insulin were measured by chemiluminescent 2-site assays (Immulite; Diagnostic Products Corp.; sensitivity = 0.2 nmol/l and 2.6 uIU/ml, respectively; intra-assay CV = 2.4 and 3.3%, respectively; inter-assay CV = 5.2 and 8.3%, respectively). Glucose was measured by the glucose oxidase method (Olympus AU 640, Olympus, Inc., Melville, NY, USA; intra-assay CV = 2.0% and inter-assay CV = 3.2%). The homeostatic index of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin using the HOMA2 Calculator v2.2.2 (http://www.dtu.ox.ac.uk/index.php?maindoc=/homa).

Statistical analysis

In order to determine the categorization of smoking based on serum cotinine levels we used a cutoff of >15 ng/ml based on the recommendations of the Society for Research on Nicotine and Tobacco (SRNT) Subcommittee report (SRNT Subcommittee on Biochemical Verification, 2002). Results are reported as mean ± standard deviation (SD) or median (25th, 75th percentiles) for continuous variables depending on the normality of the distribution. Skewed data were transformed using the natural logarithm prior to analysis. Categorical data are reported as frequencies and percentages. For the analysis of cotinine levels in the nested case–control study, contrasts were constructed from linear mixed-effects models to assess differences within each self-reported smoking group from cycle 1 to cycle 5 as well as differences between self-reported smoking groups at cycle 1. Linear mixed-effects models are an extension of regression that account for the within-subject correlation due to repeated measurements over time.

For our secondary aim of analyzing reproductive and metabolic risk factors at baseline and follow-up, we utilized the entire PPCOS I cohort of 626 subjects who have previously been described (Legro *et al.*, 2006, 2007). For this entire cohort, comparisons of these key phenotypic characteristics at baseline between the self-reported smoking groups were assessed via analysis of variance models. For analyzing changes in these phenotypic characteristics over time using the entire cohort, contrasts were constructed from linear-mixed effects models to assess differences within each self-reported smoking group from baseline to follow-up, adjusting for the randomized infertility treatment (clomiphene, metformin, or combined) and the length of time each subject remained in the study. We did not correct for BMI between groups because we found no significant differences. All hypothesis tests were two-sided and $P < 0.05$ was considered significant. SAS software, version 9.3 (SAS Institute, Inc., Cary, NC, USA) or S-plus software, version 8.1 (TIBCO Software, Palo Alto, CA, USA) was used for analyses. As this was an exploratory, rather than confirmatory, study and because the analysis was based on pre-defined hypotheses, no corrections for multiple comparisons were done (Perneger, 1998). Furthermore, we have included the 95% confidence intervals for the differences between the smoking groups to provide the readership with the precision of the estimated differences.

Results

Nested case–control study

Cycle 1 serum cotinine levels were significantly higher in self-reported current smokers compared with past smokers or never smokers ($P < 0.0001$) but past smokers had higher cotinine levels than never smokers ($P = 0.003$) (Table I and Fig. 1). Further, cotinine levels among these self-reported groups did not change over time as noted at the cycle 5 visit. Overall, self-report of smoking agreed well with smoking status as determined by measure of serum cotinine levels from cycle 1, at 90% or better for each of the groups (Table I). Five (10%) of the fifty self-reported past smokers had cotinine levels >15 ng/ml at cycle 1, and three (6%) of these women also had a raised cotinine level at cycle 5.

There were minor changes in smoking status (binary yes/no) as determined by serum cotinine levels over time, with the greatest change found in the smoking group (Fig. 2). In the never smoker group, one subject became a smoker (this outlier's sample was re-run with the same elevated result), in the past smoker group, four subjects changed status, and in the current smoker group, 7 subjects changed status.

Full randomized cohort study

We examined the association between reproductive and metabolic risk factors and self-reported smoking category at baseline in the full cohort (Table II). Self-reported never smokers have significantly lower baseline hirsutism scores than past smokers and marginally lower ($P = 0.09$) than current smokers. Never smokers weigh marginally less than current smokers ($P = 0.07$) at baseline. Current smokers had significantly higher T levels than never smokers at baseline and significantly higher hemoglobin levels than the other groups ($P < 0.0001$).

Since the nested case–control study confirmed that the initial self-report of smoking is consistent over time, we further examined changes from baseline to follow-up in the whole cohort by self-reported smoking status (Table III). Weight decreased significantly from baseline

Table I Results by self-report of smoking status at enrollment, both at baseline and follow-up study visit.

	Never ($N = 50$)		Past ($N = 50$)		Current ($N = 48$)	
	Baseline *n* **(%)**	**Follow-up** *n* **(%)**	**Baseline** *n* **(%)**	**Follow-up** *n* **(%)**	**Baseline** *n* **(%)**	**Follow-up** *n* **(%)**
Serum cotinine >15 ng/ml	0 (0%)	1 (2%)	5 (10%)	5 (10%)	44 (92%)	41 (85%)
	Median (25th, 75th percentile)	**Median (25th, 75th percentile)**	**Median (25th, 75th percentile)**	**Median (25th, 75th percentile)**	**Median (25th, 75th percentile)**	**Median (25th, 75th percentile)**
Serum cotinine (ng/ml)	0.05 (0.05, 0.20)	0.05 (0.05, 0.14)	0.08 (0.05, 0.78)	0.05 (0.05, 0.49)	135 (59, 299)	121 (39, 224)

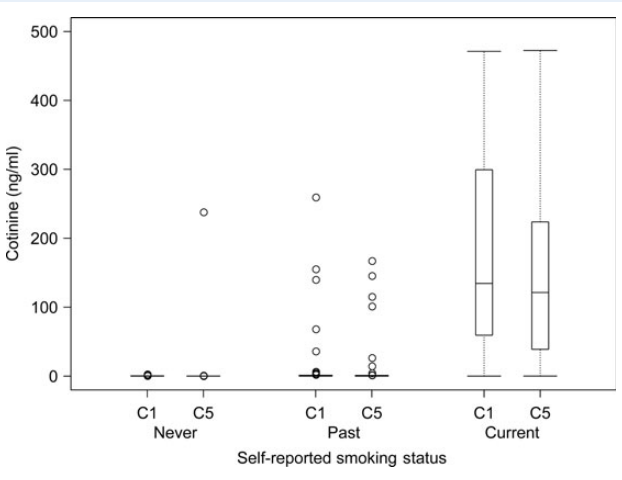

Figure 1 Cotinine levels obtained during the first (C1) and fifth (C5) cycle on study medication (box and whiskers plot displaying the median, the upper and lower quartiles, and the extreme values (outliers)).

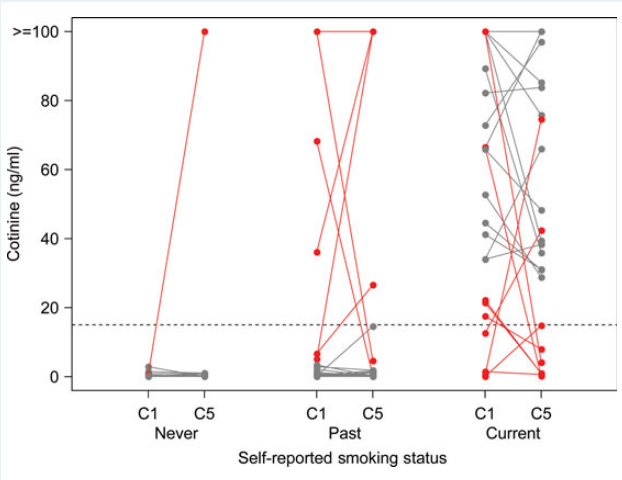

Figure 2 Change in smoking status based on serum cotinine levels from cycle 1 (C1) to cycle 5 (C5). Dotted horizontal line is the cutoff of 15 ng/ml between smokers and non-smokers. Red lines indicate individuals having at least one cotinine measurement inconsistent with their self-report. Gray lines indicated those whose cotinine measurements at both time points agreed with their self-report. Multiple subjects are represented at the >=100 mark in the past and current smoker groups.

to follow-up in never ($P = 0.002$) and past smokers ($P = 0.03$). Testosterone decreased significantly from baseline to follow-up for never and past smokers ($P < .0001$ for both) and marginally ($P = 0.08$) for current smokers; SHBG increased significantly from baseline to follow-up in all three groups. Current smokers also have a significant increase from baseline to follow-up for insulin and HOMA-IR, and this change is significant compared with never smokers.

We detected no significant association between smoking status and ovulation, though the conception and live birth rates were higher and more favorable among never smokers and past smokers than current smokers (Table IV).

Discussion

In this study, we noted that self-report of smoking as obtained by questionnaire is very accurate in self-reported never smokers and at least 90% accurate among past smokers in a group of infertile women with PCOS seeking pregnancy. As the women in this study were not specifically informed that cotinine was being measured or the accuracy of their self-reported smoking checked, we believe our results are likely generalizable to PCOS patients seeking fertility treatment. Finally, although we noted minimal differences in select reproductive and metabolic parameters according to the subject's self-report of smoking in our case–control study (data not shown), utilizing the larger full study cohort based on their self-reported categorical smoking status revealed that smokers were more hyperandrogenic at baseline and tended to have blunted improvements in reproductive parameters and worsening of metabolic factors over the course of the study compared with non-smokers. We found no association between smoking and ovulation and a trend toward more favorable pregnancy outcomes among non-smokers.

Approximately 10% of those claiming to be past smokers had serum cotinine levels indicative of recent (past week) nicotine absorption. It is possible that some of these women had absorbed nicotine from an active source other than cigarette smoking (e.g. cigar, smokeless tobacco or nicotine replacement product), but these levels were too high to be caused by environmental smoke exposure. This underlines the importance of recording a full nicotine/tobacco use history during

Table II Results of key phenotypic characteristics by self-reported smoking status at baseline in the whole PPCOS I study cohort.

	Current smoker (*N* = 107)	Past smoker (*N* = 140)	Never smoker (*N* = 379)	Current versus never	Past versus never	Current versus past
	Mean ± SD	Mean ± SD	Mean ± SD	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Age (years)	27.8 ± 3.8	28.0 ± 3.8	28.2 ± 4.1	−0.4 (−1.3 ,0.5)	−0.3 (−1.1, 0.5)	−0.1 (−1.2, 0.9)
Weight (kg)	97.6 ± 24.1	96.4 ± 25.6	92.6 ± 24.4	5.0 (−0.3, 10.3)	3.7 (−1.0, 8.5)	1.2 (−5.0, 7.4)
Waist circumference (cm)	105.2 ± 18.9	103.6 ± 18.5	101.4 ± 20.1	3.8 (−0.4, 8.0)	2.2 (−1.6, 6.0)	1.6 (−3.3, 6.6)
Hirsutism by Ferriman Gallwey Score	15.3 ± 8.2	15.4 ± 8.9	13.8 ± 7.4	1.4 (−0.2, 3.1)	1.6 (0.1, 3.1)	−0.1 (−2.1, 1.8)
	Median (25th, 75th percentile)	Median (25th, 75th percentile)	Median (25th, 75th percentile)	Ratio of geometric means (95% CI)	Ratio of geometric means (95% CI)	Ratio of geometric means (95% CI)
Testosterone (ng/dl)	60.9 (46.6, 80.4)	59.0 (43.6, 73.4)	53.3 (41.3, 74.8)	1.2 (1.0, 1.3)	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)
SHBG (nmol/l)	25.2 (15.1, 32.0)	23.6 (17.7, 25.5)	25.7 (18.1, 38.0)	0.9 (0.8, 1.0)	1.0 (0.9, 1.1)	0.9 (0.8, 1.0)
Insulin (μU/ml)	15.7 (10.1, 23.8)	15.1 (9.4, 26.5)	16.8 (9.8, 27.6)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.8, 1.2)
HOMA-IR	2.1 (1.5, 4.2)	2.0 (1.3, 3.8)	2.1 (1.2, 3.4)	1.0 (0.8, 1.2)	1.0 (0.9, 1.2)	1.0 (0.8, 1.2)
Hemoglobin (g/dl)	14.2 (13.4, 14.9	13.9 (13.4, 14.5)	13.7 (12.9, 14.4)	1.04 (1.02, 1.05)	1.02 (1.01, 1.04)	1.01 (0.99, 1.04)

Table III Changes at follow-up visit by self-reported smoking status at baseline in the whole PPCOS I cohort.

	Current smoker (*N* = 107)	Past smoker (*N* = 140)	Never smoker (*N* = 379)	Current versus never	Past versus never	Current versus past
	Mean difference (95% CI) [*P*-value]	Mean difference (95% CI) [*P*-value]	Mean difference (95% CI) [*P*-value]	*P*-value	*P*-value	*P*-value
Weight (kg)	0.1 (−0.8, 1.0) [0.85]	−0.9 (−1.7, −0.1) [0.03]	−0.8 (−1.3, −0.3) [0.002]	0.11	0.74	0.11
Waist circumference (cm)	−0.7 (−2.4, 1.1) [0.46]	−0.8 (−2.3, 0.8) [0.34]	−0.9 (−1.9, 0.0) [0.06]	0.80	0.87	0.94
	Ratio of geometric means (95% CI) [*P*-value]	Ratio of geometric means (95% CI) [*P*-value]	Ratio of geometric means (95% CI) [*P*-value]	*P*-value	*P*-value	*P*-value
Testosterone (ng/dl)	0.9 (0.8, 1.0) [0.08]	0.8 (0.8, 0.9) [<.0001]	0.8 (0.8, 0.9) [<.0001]	0.07	0.88	0.16
SHBG (nmol/l)	1.4 (1.3, 1.5) [<.0001]	1.3 (1.2, 1.4) [<.0001]	1.3 (1.3, 1.4) [<.0001]	0.35	0.76	0.30
Insulin (μU/ml)	1.2 (1.1, 1.4) [0.003]	1.0 (0.9, 1.2) [0.75]	1.0 (0.9, 1.1) [0.68]	0.02	0.95	0.05
HOMA-IR	1.3 (1.1, 1.5) [0.001]	1.0 (0.9, 1.2) [0.47]	1.0 (0.9, 1.1) [0.96]	0.002	0.52	0.04
Hemoglobin (g/dl)	0.96 (0.95, 0.97) [<.0001]	0.96 (0.95, 0.97) [<.0001]	1.01 (0.99, 1.04) [<.0001]	0.40	0.06	0.48

Mean difference constructed as follow-up minus baseline; ratio of geometric means constructed as follow-up/baseline.
Analysis adjusted for the randomized infertility treatment and the number of days each subject remained in study.

a clinical assessment. In this study smoking habits tended to be consistent over time, without clear evidence of higher recidivism rates among self-reported past smokers.

Our findings hold several important practice implications. First, because the self-report of smoking is generally accurate in this representative population, additional resources and time should be focused on

Table IV Chance of ovulation, conception (defined as positive serum human chorionic gonadotrophin) and live birth by smoking status.

	Current smoker	Past smoker	Never smoker	Current versus never		Past versus never		Current versus past	
	N (%)	*N* (%)	*N* (%)	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
Ovulation	77 (72.0)	98 (70.0)	271 (71.5)	1.02 (0.63, 1.65)	0.93	0.93 (0.61, 1.42)	0.74	1.10 (0.63, 1.92)	0.74
Conception	23 (21.5)	40 (28.6)	104 (27.4)	0.72 (0.43, 1.21)	0.22	1.06 (0.69, 1.63)	0.80	0.68 (0.38, 1.23)	0.21
Live birth	14 (13.1)	30 (21.4)	74 (19.5)	0.62 (0.33, 1.15)	0.13	1.12 (0.70, 1.81)	0.63	0.55 (0.28, 1.10)	0.09

those infertile women who acknowledge current smoking to achieve smoking cessation. In a recent randomized trial, a low impact intervention resulted in higher smoking cessation rates among infertile women compared with pregnant women (Hughes *et al.*, 2000). Thus, this population may be more receptive to intervention prior to pregnancy than during pregnancy. Second, self-reported never smokers may be held to their word when it comes to current smoking, and it is unlikely that they will commence smoking during their infertility treatment. However, a small proportion of self-reported past smokers (10% in the present study) may be expressing an aspiration rather than an accurate description of their current smoking. It may be more informative to ask patients, 'have you smoked or used a nicotine containing product in the past month?' rather than asking 'do you currently smoke cigarettes?' Those who have used a nicotine product in the past month may be considered at higher risk for continued use, as was observed in five cases in this study (three had elevated cotinine levels at both time points, and two additional cases had elevated levels at the later time point only).

Our study has several weaknesses. Selection bias may have altered our results in that we selectively studied subjects who had at least two study visits on medication, and thus we were less likely to include study drop-outs or study completers due to early pregnancy. Additionally it is retrospective and we collected only limited history on smoking, use of other nicotine products and environmental exposures. It is possible that some subjects may have been classified into categories of current smoking based on elevated cotinine levels from second hand smoke exposure, though our cutoff was chosen based on the SRNT cutoff to avoid misclassification of second hand smokers as smokers. However, it appears that exposure to second hand smoke is common among pregnant women, reaching 22% among women in Canada (Wong *et al.*, 2013), though we do not have similar data for infertile women.

This study was underpowered to look at the effects of smoking on reproductive outcomes. We have previously found that the self-report of smoking is not a predictive factor for ovulation and pregnancy (Rausch *et al.*, 2009) and report here the data in detail. However, in this study which included the whole cohort we found that current smoking is associated with a more severe phenotype at baseline and a lessened response to treatment in terms of metabolic and reproductive risk factors. In addition to a decreased chance for a successful pregnancy, women with PCOS who smoke have a worsened metabolic profile, consistent with previous studies (Glintborg *et al.*, 2012; Pau *et al.*, 2013). Hemoconcentration (as indicated by the higher hemoglobin levels in the current smokers) also likely raises serum levels of metabolic factors associated with insulin resistance (Bachen *et al.*, 2002).

We found that self-report of smoking accurately corresponds to cotinine levels in blood. Past smokers do have a substantial current smoking rate at 10%, so this category may benefit from additional counseling. A history of smoking, past or current, suggests greater lability in smoking cessation/re-initiation over time while participating in infertility treatment. Future studies may benefit from more accurate collection of smoking/nicotine use history, both active and passive, as well as updating that self-report throughout the study. A targeted intervention of smokers both prior to and during infertility therapy can be designed based on self-report rather than screening for the presence of cotinine in urine and blood.

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Authors' roles

R.S.L., G.C., A.R.K. and J.F. designed the study. W.D.S., M.P.D., C.C., S.A.C., M.P.S., B.R.C., P.G.M., N.A.C., G.G.G., J.E.N. and R.S.L. performed the PPCOS I study, A.R.K., E.R.M., and H.Z. performed data analysis and coordination for the study. R.S.L. wrote the paper and had primary responsibility for final content and all authors read and approved the paper.

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Conflict of interest

None declared.

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