Effect of exposure to second-hand smoke from husbands on biochemical hyperandrogenism, metabolic syndrome and conception rates in women with polycystic ovary syndrome undergoing ovulation induction

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ABSTRACT

STUDY QUESTION: Does second-hand smoke (SHS) exposure from husbands have adverse effects on sex hormones, metabolic profiles, clinical phenotypes and fertility outcomes in women with polycystic ovary syndrome (PCOS) undergoing ovulation induction?

SUMMARY ANSWER: SHS exposure is associated with worsened biochemical hyperandrogenism, higher incidence of metabolic syndrome and reduced conception rates in women with PCOS.

WHAT IS KNOWN ALREADY: Smoking in women impairs fecundity at some stages of reproductive process including folliculogenesis, embryo transport, endometrial angiogenesis, and uterine blood flow. Yet little is known about the hazard of SHS exposure in women with PCOS.

STUDY DESIGN, SIZE, DURATION: Secondary analysis of the Polycystic Ovary Syndrome Acupuncture and Clomiphene Trial (PCOSAct), a large randomized controlled trial conducted at 27 hospitals from 2012 to 2015 in mainland China.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Out of 1,000 women with PCOS, SHS exposure status were available in 500 women, of whom 271 women were non-exposed, and 229 exposed to cigarette smoke (170 ≤10 cigarettes per day as low-SHS exposed and 59 >10 cigarettes per day as high-SHS exposed). We compared circulating sex steroids, glucose and lipid metabolism, metabolic syndrome and phenotypes, fertility and obstetric outcomes between non-exposed and exposed women.

MAIN RESULTS AND THE ROLE OF CHANCE: Women exposed SHS, compared to non-exposed women, had a higher serum total testosterone (1.7 vs 1.5 nmol/L, P=0.01), free androgen index (5.7 vs 4.0, P=0.001) and lower sex hormone binding globulin (30.1 vs 35.6 nmol/L, P=0.03). Metabolic syndrome, but not other phenotypes, was more frequent in exposed women as compared to non-exposed women (21.8% vs 13.3%, adjusted OR=1.66; 95%CI, 1.02-2.71, P=0.04). Ovulation rates between exposed and non-exposed groups were not significantly different (76.9% vs 82.9%, adjusted OR=0.72; 95%CI, 0.45-1.15, P=0.17). Conception rates were significant lower in exposed group (26.6% vs 36.9%; adjusted OR=0.61; 95%CI, 0.41-0.91; P=0.01), while clinical pregnancy and live birth rates showed a similar trend that was not significantly different. Gestational age, birth weight and other obstetric outcomes were not affected by SHS exposure.

LIMITATIONS, REASONS FOR CAUTION: Data on SHS exposure were missing in 50% of the women. We did not assay serum nicotine or cotinine levels to quantify the SHS exposure status.

WIDER IMPLICATIONS OF THE FINDINGS: These data suggest that smoking partners from infertile women with PCOS who seek treatment should be advised to quit smoking.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a prevalence range of 5% to 15% (Rosenfield and Ehrmann, 2016). It is characterized by infertility, menstrual dysfunction, and hirsutism. Other manifestations include metabolic abnormalities, insulin resistance, dyslipidemia, type 2 diabetes mellitus and cardiovascular diseases (Gu et al., 2009; Pau et al., 2013).

It is well known that smoking is associated with many diseases. including infertility (Practice Committee of the American Society for Reproductive Medicine, 2012). In women, smoking is likely to impair the fecundity in some stages of reproductive process (Dechanet et al., 2010). It was also reported to be associated with low birth weight in pregnancies of women who smoked while undergoing assisted reproductive technology (ART) (Tong et al., 2016). Exposed to second-hand smoke (SHS) was associated with an increased risk of implantation failure and lower live birth rate in infertile women undergoing ART (Benedict et al., 2011). For women with PCOS, smoking could result in aggravated hyperandrogenism and insulin resistance, and increased risk of metabolic syndrome (Cupisti et al., 2010; Pau et al., 2013). How a habitual smoking partner impacts on the reproductive parameters in women with PCOS? Compared with non-smokers, the couples in which both partners smoked had a lower chance of live birth (OR = 0.20; 95%CI, 0.08-0.52), but no effect of female or male smoking alone (Polotsky et al., 2015). It is not clear whether the women with PCOS who smoker exposed to SHS from partner has any adverse effects on other pregnancy outcomes.

In China, the percentage of women exposed to SHS is almost 70%, while in the United States this is just under 50% (Yao et al., 2012; Fischer and Kraemer, 2017). It is therefore important to know the potential hazard of SHS exposure on endocrine, metabolism and reproduction in women with PCOS. In this study, we aimed to investigate in women with PCOS the influence of SHS exposure on circulating sex steroids and gonadotropins, glucose and lipid metabolism, metabolic syndrome and phenotypes (namely hirsutism, acne, oligomenorrhea, amenorrhea, and polycystic ovaries), as well as on fertility and obstetric outcomes.

Materials and methods

Participants

This is a prospective observational study on SHS exposure in women with PCOS. Data were recorded in the context of the PolyCystic Ovary Syndrome Acupuncture plus Clomiphene Trial (PCOSAct) (Wu et al., 2017). PCOSAct was a large-sample, multi-center, randomized controlled trial which was carried out between 2012 and 2015 in mainland China. The institutional review boards at the local sites approved the protocol and all patients together with their husbands provided written informed consent before joining the study.

The trial was registered on ClinicalTrial.gov (NCT01573858) and chictr.org.cn (ChiCTR-TRC-12002081). The study protocol has been described elsewhere (Kuang et al., 2013) and the main results were published in details (Wu et al., 2017). In short, Chinese women suffering from anovulation related to PCOS and requesting ovulation induction were eligible. PCOS was defined by modified Rotterdam criteria (Rotterdam 2004). Exclusion criteria included other endocrine disorders, use of hormonal or other medication including Chinese herbal prescriptions in the past 2 months, miscarriage or given birth within 6 weeks, and breastfeeding within the last 6 months. For the present study, husbands of the women with poor sperm counts (sperm concentrations $<15\times10^6$/ml, total motility <40%, semen volume ≤1.5ml, and total motile sperm count ≤9 million (Cooper et al., 2010)) were excluded. Women who were current smokers and/or alcohol users were excluded from the present study.

SHS exposure

SHS exposure status was classified based on a self-reported questionnaire. SHS exposure was defined as regularly exposed to SHS (lived with a partner who was a chronic smoker on a daily basis) in the past six months (Soldin et al., 2011). High- and low-exposures were further defined as a partner smoking >10 cigarettes/day and ≤10 cigarettes/day, respectively (Olivo-Marston et al., 2009). We did not assess duration of SHS per day nor assay serum nicotine or cotinine levels to determine SHS severity in this study.

Physical examination

Participating women underwent a complete physical examination at baseline including weight and height measurement, waist and hip circumferences, systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate, and transvaginal ultrasound examination of the ovaries. The body mass index (BMI) and waist-to-hip ratio (WHR) were calculated.

Interventions

In the PCOSAct, all participants were randomized to one of 4 treatments including active acupuncture plus clomiphene, control acupuncture plus clomiphene, active acupuncture plus clomiphene placebo, or control acupuncture plus clomiphene placebo for 4 menstrual cycles. Pregnancy outcomes were assessment after each cycle. Once conception was achieved, intervention was stopped and the pregnancy was followed up until miscarriage or delivery.

Biochemical measurements

Baseline fasting circulating sex steroids and gonadotropins, glucose and lipid metabolism including total testosterone (TT), free testosterone (FT), sex hormone binding globulin (SHBG), estradiol (E_2), progesterone (P), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), fasting glucose, total fasting insulin, total cholesterol, triglyceride, lipoproteins A, high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB) were measured at the core laboratory in Heilongjiang University of Chinese Medicine. All sex hormones, except FT measured by radioimmunoassay (RIA), were analyzed by electro-chemiluminescent immunoassays. Glucose and lipid profiles were measured by enzymatic methods. Free androgen index (FAI), testosterone/estradiol ratio (T/E2) and the homeostasis model assessment-insulin resistance (HOMA-IR) were calculated. Husbands' semen analyses were performed in local laboratories and semen parameters were determined according to WHO 2010 criteria (World Health Organization, 2010).

Metabolic syndrome and phenotypes

Metabolic syndrome was defined according to the ATP III criteria when 3 out of the 5 following criteria presented: waist circumference >88cm, triglycerides ≥150mg/dL, HDL <50mg/dL, blood pressure ≥135/85mmHg and fasting glucose ≥110mg/dL (Grundy et al., 2004). Hirsutism was scored in accordance

with the modified Ferryman–Gallwey (MF-G) score. MF-G score ≥5 was classified as hirsutism in Mainland China (Zhao et al., 2011). Biochemical hyperandrogenism was defined as an androstenedione level of >10.8nmol/l or total testosterone ≥2.81 nmol/L (Li et al., 2013). Acne was measured using a standard acne lesion assessment diagram and definitions (Tan et al., 2007). Oligomenorrhea was defined as an intermenstrual interval >35 days and <8 menstrual bleedings in a year, and amenorrhea was defined as an intermenstrual interval >90 days (Norman et al., 2007). Polycystic ovaries were diagnosed by transvaginal ultrasound when at least one ovary had a volume of >10ml or there were 12 or more follicles measuring 2–9mm in diameter (Balen et al., 2003).

Fertility outcomes

Ovulation was defined as a serum progesterone level above 5 ng/ml during a cycle. Ovulation rates were calculated from patients achieved one or more times ovulation in four cycles. Conception was defined as any positive serum human chorionic gonadotropin test, ie. biochemical pregnancy. Clinical pregnancy was defined as an intrauterine pregnancy with fetal heart pulsation as detected by transvaginal ultrasound. Pregnancy losses included miscarriage or fetal demises and stillbirths before and after 20 weeks, respectively. Live birth was defined as the delivery of a viable infant.

Obstetric outcomes

Obstetric outcomes including gestational age, preterm delivery, neonatal gender, birth weight, body length, 1 and 5 Apgar scores, and neonatal intensive care unit (NICU) admission rate were recorded.

Statistical Analysis

The sample size was based on the PCOSAct (Wu et al., 2017). In 500 couples, our sample size allowed us to show a difference of 10% conception rate between non-exposed and SHS exposed groups with Power 95% and Type I error 0.05.. Data were described as frequencies and percentages for categorical data, or medians (Q1, Q3) for numerical data. Mann–Whitney test and chi-square test or Fisher's exact test were used to compare differences between two groups. Differences and odds ratio (OR) for pregnancy and obstetric outcomes were estimated by multivariable logistic regression with adjustment for parameters based on the significant differences in univariate analyses. Since clomiphene, but not acupuncture, increased live birth in our PCOAct, the analysis was also adjusted by clomiphene treatment

only. The risks of outcomes between low or high SHS exposure and non-exposed group were also calculated. The interaction between clomiphene and SHS was analyzed by general liner model or logistic regression. Binary logistic regression were employed to analyze whether SHS exposure is independent from ovulation and metabolic syndrome. All hypothesis tests were two-sided. A P-value <0.05 was considered to indicate statistical significance. All statistical analyses were done using the SPSS statistical package version 22.0.

Results

Out of the 1000 women recruited in PCOSAct, all women finished the questionnaire. Yet not all couples report the smoking status, data on SHS exposure status were available in 500 women only. We excluded 12 women who were current smokers. Amongst 500 women, 271 were non-exposed, and 229 exposed, of whom 59 were high-exposed and 170 were low-exposed. The baseline age, height, weight, waist, hip, pulse, SBP, DBP, BMI, WHR of included women with PCOS, and their partners' semen parameters were comparable (**Table 1**).

Exposed women, compared to non-exposed women, had significantly higher serum total testosterone, free androgen index and lower sex hormone binding globulin (**Table 2**). There was a dose-response effect, with women in high-exposed group, but not in low-exposed group, had significantly higher total testosterone, free testosterone, free androgen index and T/E2 ratio and lower sex hormone binding globulin than those in non-exposed group.

The baseline fasting circulating glucose and insulin did not differ between the two groups. The total cholesterol, triglyceride, lipoproteins A, HDL, LDL, ApoA1, and ApoB levels were not statistically different between the exposed and non-exposed groups, nor between the high- or low-exposed and non-exposed groups (**Table 3**).

There were no differences in the proportion of hirsutism, acne, oligomenorrhea, amenorrhea, and polycystic ovary morphology between the exposed and non-exposed groups, nor between the high- or low-exposed and non-exposed groups (**Table 4**). The rate of metabolic syndrome was significant higher in women in exposed group than the non-exposed. Both high- and low-exposed groups had a higher frequency of metabolic syndrome than non-exposed group.

Amongst 500 women with PCOS, 399 had ovulation (79.8%), 161 had biochemical pregnancy (32.2%), 110 had clinical pregnancy (22.0%), 58 had pregnancy loss (36.0%) and 103 had live birth (20.6%). Ovulation (OR = 6.85, 95%CI= 3.03-15.50), conception(OR = 2.72, 95%CI= 1.63-4.46), clinical pregnancy (OR = 2.36, 95%CI= 1.33-4.18) and live birth (OR = 2.26, 95%CI= 1.26-4.06) were significant differences

between clomiphene and placebo within non-exposed group, while only ovulation (OR = 6.45, 95%CI= 3.03-13.75) and conception (OR = 2.43, 95%CI= 1.31-4.51) were significant differences within the SHS exposed groups (Supplementary Table 1). SHS exposure was not significantly associated with ovulation, but significantly associated with metabolic syndrome (Supplementary Table 2). Ovulation rate between exposed and non-exposed groups was not significantly different. Women in exposed group had lower conception rate than those in the non-exposed group, while clinical pregnancy rate, and live birth rate were also lower, but not statistically significant. Both high- and low-exposed groups, compared with the non-exposed, had lower conception rate (**Table 5**). There was no interaction between clomiphene and SHS exposure for all pregnancy outcomes.

The gestational age, preterm birth rate, male and female rates, birth weight, body length, and NICU admission between exposed group and non-exposed group were not significantly different (**Table 6**), so were high- nor low-exposed groups. None of newborns had 1 and 5 minute Apgar score <5.

Discussion

We evaluated in women with PCOS the impact of SHS exposure on circulating sex steroids, glucose and lipid metabolism, metabolic syndrome and phenotypes, fertility and obstetric outcomes. We demonstrate that women exposed to SHS have higher serum TT, FAI and lower SHBG than non-exposed women. With high-exposure, women had not only the higher serum TT and FT and lower SHBG, but also lower estradiol. Exposed to SHS is also associated with an increased risk of metabolic syndrome and lower conception rate in women with PCOS, even after adjusted for TT, FT, SHBG and treatment.

Our study thoroughly investigated the effect of SHS exposure on endocrine, metabolic, fertility and also obstetric outcomes in women with PCOS. Over 50% of SHS exposure status was not available due to incomplete questionnaire and lose of contact. Although the sample size is not very big, we have similar number of women in non-exposed and SHS exposed groups and the interventions were randomised. The potential selection bias might underestimate the adverse effects of SHS exposure, but we expected it very minimal.. We did not record the duration of SHS exposure per day nor quantify the serum nicotine/cotinine to validate the status of SHS exposure. In addition, the intercourse frequency may be a confounding factor to the pregnancy outcomes. Current evidence showed that smoking could result in erectile dysfunction (Lam et al., 2006), reduced intercourse frequency (Yamamura, 2014) and female sexual dysfunction (Choi et al., 2015), while more frequent intercourse was associated with a greater chance of successful infertility treatment (Polotsky et al., 2015). Therefore, apart from physical activity and dietary habits, smoking in men was likely to have lower intercourse frequency which, to some extent, has influence on pregnancy outcomes for infertile couples.

Tobacco smoke has a complex mixture containing an estimated 5000 chemicals (Borgerding and Klus, 2005). SHS contains most of the toxic carcinogens from tobacco smoke, nicotine is a most common and important component of SHS (Besaratinia and Pfeifer, 2008). Ovary is the major organ of testosterone biosynthesis in women. Nicotine was shown to inhibit the aromatase activity (Nelson and Bulun, 2001). Aromatase is a key enzyme responsible for the conversion of androgen to estrogen in ovary. In vitro, aromatase activity inhibited directly by nicotine, and estrogen synthesis was hampered in granulosa cells

exposed to high dose of nicotine (Barbieri et al., 1986; Sanders et al., 2002). Nicotine could also block aromatase activity in female baboons in vivo (Biegon et al., 2010). In our study, women under high SHS exposure has minimal lower estradiol levels than non-exposed (186.8 vs 194.8 pmol/L). this suggests the even high SHS exposure was still not that high as in vitro and animal studies. The sex hormone alteration effect of SHS exposure on the women with PCOS seems to be a dose response effect as the high- but not the low-exposed. We speculated a threshold effect for SHS exposure rather than dose-response effect. In a large-scale study, though it indicated that the elevation of T and FT was associated with dose-exposure of tobacco smoke in postmenopausal women (Brand et al., 2011). Unlike active smoking, the serum nicotine concentrations altered little between the passive-smokers and non-smokers (Pau et al., 2013), this may interpret in part slightly increased biochemical hyperandrogenism in low-exposed group. Here, we were not able to analyze this association because of lacking precise data regarding dose-exposure of SHS.

Our data suggest that smoking partners from infertile women with PCOS who seek treatment should be advised to quit smoking, or at least not expose their partner to second-hand smoke. Smoking cessation has substantial short and long term health benefit for smokers of all ages. Compared with those continued smoking, there was 36% reduction in relative risk of mortality of coronary heart disease (Critchley and Capewell, 2003) and 30%-50% reduced risk of lung cancer over 10 years for patients who quit (Edwards, 2004). Stop smoking can also restore the chance of pregnancy to the level of a non-smoking female, normalize the low testosterone level (Fredricsson and Gilljam, 1992) and improve erectile dysfunction in male smokers (Pourmand et al., 2004). The major barriers to quit smoking during the pregnancy of partners largely attributed to unware of passive smoking adverse effect on the fetus (Wakefield et al., 1998). Though education (Prochaska et al., 1993; Strecher et al., 2008) plus individualized smoking cessation (Fiore et al., 1990) approaches can better help primary smoker to quit smoking, partner support may be needed for successful quitting (Mermelstein and Lichtenstein, 1983).

In summary, biochemical, but not clinical hyperandrogenism, was exaggerated; metabolic syndrome was more common; and conception rate was lower in women with PCOS exposed to SHS from partners.

Authors' roles

Xiao-Ke Wu: Contributions to conception and design. Jian Li: Analysis the data and drafting the article. Ricky Qi Wu: Analysis and interpretation of data. Zhong-Ming Zhou, Ping Fu, Xiu-Hua Chen, Ying Yan, Xin Wang, Zheng-Wang Yang, Wei-Li Li, and PCOSact Study group: Acquisition of the data. Chi Chiu Wang: Revising the article critically for improtant intellectual content. Elisabet Stener-Victorin, Richard S. Legro, Ernest H. Y. Ng, Heping Zhang and Ben W. Mol: Final revision and approval of the version. The final manuscript and order of authorship has been approved by all authors.

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Conflict of interest

None declared.

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Table 1 Baseline demographic characteristics of women with PCOS and semen analysis of their partners

	Non-exposed (N=271)	SHS exposed (N=229)			P-value		
		Total (N=229)	Low (N=170)	High (N=59)	P_1	P_2	P_3
Demographic characteristics							
Age (years)	28.0 (26.0, 30.0)	28.0 (25.0, 30.0)	27.5 (25.0, 30.0)	28.0 (26.0, 30.0)	0.71	0.57	0.84
Weight (kg)	61.0 (55.0, 70.0)	60.8 (54.0, 70.0)	60.0 (54.0, 69.0)	65.0 (56.5, 75.0)	0.70	0.23	0.15
Height (cm)	160.0 (158.0,165.0)	161.0 (158.0,165.0)	160.0 (158.0, 165.0)	163.0 (158.0,166.0)	0.65	0.78	0.10
Body Mass Index (kg/m^2)	23.8 (21.1, 26.6)	23.4 (21.2, 26.3)	23.2 (20.8, 25.9)	24.4 (21.6, 27.6)	0.46	0.16	0.36
Waist (cm)	84.1 (77.0, 92.0)	84.0 (78.0, 92.0)	83.3 (77.0, 90.3)	86.0 (80.0, 94.0)	0.92	0.47	0.10
Hip (cm)	98.0 (92.0, 104.5)	97.0 (93.0, 102.0)	97.0 (92.0, 101.3)	98.0 (94.0, 104.0)	0.52	0.21	0.39
Waist to Hip Ratio	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.27	0.73	0.05
Pulse rate (beat/min)	76.0 (72.0, 80.0)	75.0 (72.0, 79.0)	75.0 (72.0, 79.0)	76.0 (72.0, 79.0)	0.14	0.10	0.66
Systolic Blood Pressure (mmHg)	110.0 (110.0,120.0)	110.0 (105.0,120.0)	110.0 (105.0,120.0)	110.0 (110.0,120.0)	0.97	0.83	0.75
Diastolic Blood Pressure (mmHg)	75.0 (70.0, 80.0)	75.0 (70.0, 80.0)	75.0 (70.0, 80.0)	72.0 (70.0, 80.0)	0.53	0.77	0.34
Semen analysis							
Volume (ml)	3.0 (2.5, 4.0)	3.0 (2.2, 4.0)	3.0 (2.4, 4.0)	3.0 (2.0, 3.6)	0.21	0.47	0.11
Concentration (million/ml)	71.2 (43.0, 103.9)	72.0 (38.1, 109.8)	72.5 (39.9, 111.9)	65.0 (30.7, 103.2)	0.67	0.93	0.38
Motility (grade a+b+c)	71.0 (58.1, 82.1)	70.0 (58.0, 79.0)	70.0 (57.0, 79.3)	67.7 (58.4,78.7)	0.16	0.28	0.20

Medians (Q1, Q3) are presented. a=progressive motility, b=non-linear motility, c=non-progressive motility. P_1=total exposed vs non-exposed, P_2=low-exposed vs non-exposed, P_3=high-exposed vs non-exposed. No significant differences between total, high, or low exposed groups and non-exposed group.

421 Table 2 Sex hormones

Sex hormones	Non-exposed (N=271)	SHS exposed (N=229)			P-value		
		Total (N=229)	Low (N=170)	High (N=59)	P_1	P_2	P_3
Luteinizing hormone (IU/L)	9.2 (6.0, 13.8)	9.6 (6.6, 14.4)	10.0 (6.4, 14.8)	9.0 (6.7, 13.4)	0.31	0.26	0.82
Follicle Stimulating hormone (IU/L)	6.0 (4.9, 7.1)	6.1 (5.1, 7.0)	6.1 (5.1, 6.9)	6.2 (5.2, 7.5)	0.46	0.75	0.24
Progesterone (nmol/L)	1.7 (1.2, 2.4)	1.8 (1.3, 2.4)	1.8 (1.3, 2.6)	1.8 (1.2, 2.3)	0.40	0.42	0.63
Estradiol (pmol/L)	194.8 (154.9, 249.9)	201.0 (161.1, 266.9)	214.4 (165.4, 273.8)	186.8 (156.3, 233.5)	0.20	0.05	0.40
Total testosterone (nmol/L)	1.5 (1.1, 1.9)*	1.7 (1.2, 2.1)	1.6 (1.2, 2.1)	1.8 (1.5, 2.1)#	0.01	0.10	0.005
Free Androgen Index	4.0 (2.3, 6.8)*	5.7 (3.0, 8.7)	4.7 (2.6, 8.2)	7.2 (4.9, 10.2)#	0.001	0.08	<0.001
Free testosterone (pmol/L)	7.5 (5.8, 9.6)	7.8 (5.8, 10.0)	7.5 (5.7, 9.2)	9.4 (7.0, 12.1)#	0.28	0.56	<0.001
Testosterone to Estradiol ratio	7.7 (5.6, 10.0)	7.9 (5.8, 10.2)	7.6 (5.5, 9.6)	9.0 (6.8, 12.1)#	0.58	0.45	0.005
Sex hormone binding globulin (nmol/L)	35.6 (23.5, 59.4)*	30.1 (19.3, 52.8)	33.7 (20.0, 60.7)	25.0 (18.0, 42.1)#	0.03	0.36	<0.001

422 Medians (Q1, Q3) are presented. P_1=total exposed vs non-exposed, P_2=low-exposed vs non-exposed, P_3=high-exposed vs non-exposed, *p<0.05
423 compared total exposed with non-exposed groups, #p<0.05 compared high-exposed with non-exposed groups.

Table 3 Glucose and lipid metabolism

Glucose and Lipid	Non-exposed (N=271)	SHS exposed (N=229)			P-value		
		Total (N=229)	Low (N=170)	High (N=59)	P_1	P_2	P_3
Total fasting insulin (pmol/L)	71.4 (47.4, 119.8)	72.0 (48.6, 115.8)	69.6 (47.2, 107.7)	86.8 (52.1,157.8)	0.62	0.82	0.10
Fasting glucose (mmol/L)	5.0 (4.6, 5.5)	5.1 (4.5, 5.6)	5.2 (4.5, 5.6)	5.1 (4.5, 5.5)	0.26	0.25	0.64
HOMA-IR	2.6 (1.6, 4.3)	2.6 (1.8, 4.5)	2.5 (1.7, 4.1)	2.9 (2.1, 5.8)	0.62	0.82	0.10
Total cholesterol (mmol/L)	4.6 (4.0, 5.3)	4.7 (4.0, 5.5)	4.7 (4.0, 5.5)	4.6 (3.9, 5.3)	0.44	0.28	0.79
Triglyceride (mmol/L)	1.3 (1.0, 1.8)	1.4 (1.0, 2.0)	1.3 (1.0, 1.9)	1.7 (1.1, 2.2)	0.39	0.86	0.08
Lipoproteins A (g/L)	0.10 (0.07, 0.15)	0.10 (0.07, 0.14)	0.10 (0.07, 0.15)	0.10 (0.07, 0.15)	0.99	0.96	0.90
High-density lipoprotein (mmol/L)	1.2 (1.0, 1.5)	1.2 (1.0, 1.5)	1.2 (1.0, 1.5)	1.2 (1.0, 1.4)	0.69	0.89	0.23
Low-density lipoprotein (mmol/L)	2.9 (2.3, 3.4)	3.0 (2.5, 3.5)	3.0 (2.5, 3.6)	2.9 (2.4, 3.3)	0.24	0.18	0.84
Apolipoprotein A1 (g/L)	1.5 (1.3, 1.7)	1.5 (1.3, 1.7)	1.5 (1.3, 1.7)	1.4 (1.2, 1.6)	0.63	0.81	0.10
Apolipoprotein B (g/L)	0.9 (0.7, 1.0)	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	0.15	0.27	0.17

Medians (Q1, Q3) are presented. P1=total exposed vs non-exposed, P2=low-exposed vs non-exposed, P3=high-exposed vs non-exposed. No significant differences between total, high, or low exposed groups and non-exposed group.

427 Table 4 Clinical Phenotypes

Phenotypes	Non-exposed (N=271)	SHS exposed (N=229)			Total vs non-exposed			Low vs non-exposed			High vs non-exposed		
		Total (N=229)	Low (N=170)	High (N=59)	Non-adjusted OR; 95% CI	Adjusted OR; 95% CI	P-value	Non-adjusted OR; 95% CI	Adjusted OR; 95% CI	P-value	Non-adjusted OR; 95% CI	Adjusted OR; 95% CI	P-value
Hirsutism	74 (27.3)	60 (26.2)	39 (22.9)	21 (35.6)	0.95 (0.64-1.41)	0.91 (0.61-1.36)	0.65	0.79 (0.51-1.25)	0.75 (0.48-1.18)	0.21	0.95 (0.64-1.41)	1.35 (0.73-2.51)	0.33
Acne	84 (31.0)	67 (29.3)	49 (28.8)	18 (30.5)	0.92 (0.63-1.35)	0.87 (0.59-1.28)	0.47	0.90 (0.59-1.38)	0.86 (0.56-1.33)	0.51	0.99 (0.55-1.80)	0.88 (0.46-1.65)	0.68
Oligomenorrhea	234 (86.4)	196 (85.6)	145 (85.3)	51 (86.4)	0.94 (0.5-1.56)	1.02 (0.61-1.72)	0.93	0.92 (0.54-1.57)	0.93 (0.53-1.63)	0.80	1.01 (0.45-2.17)	1.32 (0.56-3.11)	0.52
Amenorrhea	33 (12.2)	33 (14.4)	25 (14.7)	8 (13.6)	1.22 (0.72-2.04)	0.97 (0.58-1.63)	0.93	1.24 (0.71-2.18)	1.08 (0.61-1.89)	0.80	1.13 (0.52-2.60)	0.76 (0.32-1.78)	0.52
Polycystic ovaries	218 (80.4)	187 (81.7)	139 (81.8)	48 (81.4)	1.08 (0.69-1.70)	1.07 (0.68-1.69)	0.77	1.09 (0.67-1.80)	1.14 (0.69-1.88)	0.60	1.06 (0.52-2.21)	0.86 (0.40-1.85)	0.69
Metabolic syndrome	36 (13.3)	50* (21.8)	36# (21.2)	14 (23.7)	1.82 (1.13-2.95)	1.66 (1.02-2.71)	0.04	1.75 (1.05-2.92)	1.68 (0.99-2.86)	0.05	2.03 (0.99-4.09)	1.62 (0.77-3.39)	0.20

428 n (%) are presented. Adjusted for total testosterone, free testosterone, sex hormone binding globulin and clomiphene. *p<0.05 compared total
429 exposed with non-exposed groups, #p<0.05 compared low-exposed with non-exposed groups.

Table 5 Ovulation and pregnancy outcomes

Ovulation and Pregnancy outcomes	Non-exposed (N=271)	SHS exposed			Total vs non-exposed			Low vs non-exposed			High vs non-exposed		
		Total (N=229)	Low (N=170)	High (N=59)	Non-adjusted OR; 95% CI	Adjusted OR; 95% CI	P-value	Non-adjusted OR; 95% CI	Adjusted OR; 95% CI	P-value	Non-adjusted OR; 95% CI	Adjusted OR; 95% CI	P-value
Ovulation	223 (82.9)	176 (76.9)	138 (81.2)	38 (64.4)[#]	0.72 (0.46-1.11)	0.72 (0.45-1.15)	0.17	0.93 (0.56-1.53)	0.90 (0.53-1.52)	0.69	0.39 (0.21-0.74)	0.50 (0.24-1.02)	0.06
Conception	100 (36.9)	61 (26.6)[*]	49 (28.8)	12 (20.3)[#]	0.62 (0.42-0.91)	0.61 (0.41-0.91)	0.01	0.69 (0.46-1.05)	0.66 (0.43-1.01)	0.06	0.44 (0.23-0.86)	0.48 (0.24-0.97)	0.05
Clinical pregnancy	69 (25.5)	41 (17.9)[*]	33 (19.4)	8 (13.6)	0.64 (0.41-0.99)	0.65 (0.42-1.01)	0.06	0.71 (0.45-1.12)	0.70 (0.44-1.13)	0.15	0.46 (0.22-1.02)	0.50 (0.22-1.13)	0.09
Pregnancy loss	35 (35.0)	23 (37.7)	19 (38.8)	4 (33.4)	1.12 (0.58-2.18)	0.71 (0.40-1.26)	0.24	1.18 (0.60-2.38)	0.77 (0.42-1.41)	0.41	0.93 (0.29-2.95)	0.55 (0.18-1.65)	0.28
Live birth	65 (24.0)	38 (16.6)[*]	30 (17.6)	8 (13.6)	0.63 (0.40-0.99)	0.64 (0.41-1.02)	0.06	0.68 (0.42-1.10)	0.68 (0.42-1.11)	0.12	0.50 (0.23-1.11)	0.55 (0.24-1.25)	0.15

n (%) are presented. Adjusted for total testosterone, free testosterone, sex hormone binding globulin and clomiphene. *p<0.05 compared total exposed with non-exposed groups, [#]p<0.05 compared high-exposed with non-exposed groups.

433 Table 6 Obstetric outcomes

Obstetric outcomes	Non-exposed (n=65)	SHS exposed			P-value		
		Total (n=38)	Low (n=30)	High (n=8)	P_1	P_2	P_3
Gestational age (days)	274 (266.0, 280.5)	279(270.0, 282.0)	279 (270.8, 280.3)	280 (265.3, 289.5)	0.13	0.21	0.25
Preterm delivery	9/65 (13.9)	1/38 (2.6)	1/30 (3.3)	0/8 (0)	0.09	0.16	0.58
Gender							
male	27/65 (41.5)	17/38 (44.7)	14/30 (46.7)	3/8 (37.5)	0.84	0.66	0.99
female	38/65 (58.5)	21/38 (55.3)	16/30 (53.3)	5/8 (62.5)	0.84	0.66	0.99
Birth weight (g)	3300 (2950, 3575)	3425 (3175, 3750)	3375 (3175, 3900)	3500 (2888, 3638)	0.17	0.21	0.46
Body length (cm)	50.0 (49.0, 50.0)	50.0 (50.0, 51.0)	50.0 (50.0, 51.0)	50.0 (48.0, 51.0)	0.08	0.07	0.48
1 min Apgar score < 5	0/65 (0)	0/38 (0)	0/30 (0)	0/8 (0)	NA	NA	NA
5 min Apgar score < 5	0/65 (0)	0/38 (0)	0/30 (0)	0/8 (0)	NA	NA	NA
NICU admission rate	8/65 (12.3)	1/38 (2.6)	1/30 (3.3)	0/8 (0)	0.15	0.26	0.59

434 Medians (Q1, Q3) or n/N (%) are presented. NA: not available, NICU: neonatal intensive care unit. P_1=total exposed vs non-exposed, P_2=low-
435 exposed vs non-exposed, P_3=high-exposed vs non-exposed. No significant differences between total, high, or low exposed groups and non-
436 exposed group.

437 Supplementary table 1 Ovulation and pregnancy outcomes among 4 intervention

Ovulation and Pregnancy outcomes (%)	Non-exposed (N=271)	SHS Exposed (N=229)			*P*-value		
		Total (N=229)	Low (N=170)	High (N=59)	P_1	P_2	P_3
Ovulation	223/271 (82.9)	176/229 (76.9)	138/170 (81.2)	38/59 (64.4)#	0.15	0.80	<0.01
active acupuncture plus clomiphene	64/67 (95.5)	55/58 (94.8)	39/41 (95.1)	16/17 (94.1)	>0.99	>0.99	>0.99
control acupuncture plus clomiphene	63/68 (92.6)	50/57 (87.7)	41/46 (89.1)	9/11 (81.8)	0.38	0.52	0.25
active acupuncture plus clomiphene placebo	48/67 (71.6)	34/57 (59.6)	25/37 (67.6)	9/20 (45.0)#	0.19	0.66	0.03
control acupuncture plus clomiphene placebo	48/69 (69.6)	37/57 (64.9)	33/46 (71.7)	4/11 (36.4)#	0.70	0.84	0.04
P-value$	<0.0001	<0.0001	<0.01	<0.01			
Conception	100/271 (36.9)	61/229 (26.6)*	49/170 (28.8)	12/59 (20.3)#	0.02	0.10	0.02
active acupuncture plus clomiphene	35/67 (52.2)	20/58 (34.5)	14/41 (34.1)	6/17 (35.3)	0.05	0.08	0.28
control acupuncture plus clomiphene	30/68 (44.1)	20/57(35.1)	17/46 (37.0)	3/11 (27.3)	0.36	0.56	0.34
active acupuncture plus clomiphene placebo	18/67 (26.9)	10/57 (17.5)	9/37 (24.3)	1/20 (5.0)	0.28	0.82	0.06
control acupuncture plus clomiphene placebo	17/69 (24.6)	11/57 (19.3)	9/46 (19.6)	2/11 (18.2)	0.52	0.65	>0.99
P-value$	<0.01	0.05	0.23	0.13			
Clinical Pregnancy	69/271 (25.5)	41/229 (17.9)*	33/170 (19.4)	8/59 (13.6)	0.05	0.16	0.06
active acupuncture plus clomiphene	24/67 (35.8)	12/58 (20.7)	9/41 (22.0)	3/17 (17.6)	0.08	0.14	0.24
control acupuncture plus clomiphene	21/68 (30.9)	13/57 (22.8)	11/46 (23.9)	2/11 (18.2)	0.31	0.42	0.39
active acupuncture plus clomiphene placebo	13/67 (19.4)	6/57 (10.5)	5/37 (13.5)	1/20 (5.0)	0.17	0.45	0.12
control acupuncture plus clomiphene placebo	11/69 (15.9)	10/57 (17.5)	8/46 (17.4)	2/11 (18.2)	0.81	0.84	0.85
P-value$	0.02	0.34	0.64	0.59			

Pregnancy loss	35/100 (35.0)	23/61 (37.7)	19/49 (38.8)	4/12 (33.4)	0.73	0.65	0.91
active acupuncture plus clomiphene	13/35 (37.1)	9/20 (45.0)	6/14 (42.9)	3/6 (50.0)	0.57	0.71	0.55
control acupuncture plus clomiphene	10/30 (33.3)	8/20 (40.0)	7/17 (41.2)	1/3 (33.3)	0.63	0.59	>0.99
active acupuncture plus clomiphene placebo	6/18 (33.3)	4/10 (40.0)	4/9 (44.4)	0/1 (0.0)	0.72	0.57	0.49
control acupuncture plus clomiphene placebo	6/17 (35.3)	2/11 (18.2)	2/9 (22.2)	0/2 (0.0)	0.33	0.49	0.31
P-value[$]	0.99	0.51	0.73	0.52			
Live birth	65/271 (24.0)	38/229 (16.6)*	30/170 (17.6)	8/59 (13.6)	0.04	0.12	0.08
active acupuncture plus clomiphene	22/67 (32.8)	11/58 (19.0)	8/41 (19.5)	3/17 (17.6)	0.08	0.13	0.22
control acupuncture plus clomiphene	20/68 (29.4)	12/57 (21.1)	10/46 (21.7)	2/11 (18.2)	0.28	0.36	0.44
active acupuncture plus clomiphene placebo	12/67 (17.9)	6/57 (10.5)	5/37 (13.5)	1/20 (5.0)	0.24	0.56	0.16
control acupuncture plus clomiphene placebo	11/69 (15.9)	9/57 (15.8)	7/46 (15.2)	2/11 (18.2)	0.98	0.92	0.85
P-value[$]	0.05	0.46	0.74	0.59			

*n/N (percentage) are presented, P_1=total exposed vs non-exposed, P_2=low-exposed vs non-exposed, P_3=high-exposed vs non-exposed, *p<0.05 compared total exposed with non-exposed groups, [#]p<0.05 compared high-exposed with non-exposed groups, [$]p-value of 4 intervention comparison within group.*

Supplementary table 2 Significant variables associated with ovulation and metabolic syndrome in women with PCOS[a]

Outcomes	Variables	Variable in the Equation					
		B	S.E	Wald	df	Sig.	Exp(B)
ovulation	Clomiphene	1.902	.283	45.290	1	.000	6.700
	LH	-.127	.037	11.660	1	.001	.881
	Oligomenorrhea	1.417	.447	10.038	1	.002	4.128
	Pulse	-.079	.029	7.628	1	.006	.924
	SHBG	-.023	.008	7.253	1	.007	.977
	Sperm volume	.236	.126	4.137	1	.042	1.266
Metabolic syndrome	HDL	-7.054	1.800	15.360	1	.000	.001
	Triglyceride	1.778	.396	20.125	1	.000	5.915
	LH	.162	.056	8.524	1	.004	1.176
	LDL	3.527	1.452	5.897	1	.015	34.022
	Sperm volume	-.456	.208	4.821	1	.028	.634
	SHS exposure	.997	.455	4.808	1	.028	2.709
	Age	-.141	.069	4.146	1	.042	.869

[a]*All variable(s) entered: SHS exposure, clomiphene, acupuncture, age, height, weight, body mass index (BMI), hip and waist circumferences, waist to hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse, sperm volume, concentration and motility (grade a+b+c), luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, total testosterone (TT), free testosterone (FT), estradiol, sex hormone binding globulin (SHBG), free androgen index (FAI), testosterone to estradiol ratio, total fasting insulin and glucose, homeostasis model assessment-insulin resistance (HOMA-IR), total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), lipoproteins A, hirsutism, acne, oligomenorrhea, polycystic ovaries morphology, metabolic syndrome, ovulation, conception, clinical pregnancy, pregnancy loss, and live birth. Only significant variables are presented.*