


BMJ Open Prevalence and characteristics of polycystic ovary syndrome in Brazilian women: protocol for a nation-wide case-control study

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To cite: Spritzer PM, Marchesan LB, Santos BR, *et al*. Prevalence and characteristics of polycystic ovary syndrome in Brazilian women: protocol for a nation-wide case-control study. *BMJ Open* 2019;**9**:e029191. doi:10.1136/bmjopen-2019-029191

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2019-029191>).

Received 18 January 2019
Revised 05 August 2019
Accepted 13 August 2019



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ABSTRACT

Introduction Brazil is a large country, with a population of mixed ethnic background and broad variation in dietary and physical activity traits across its five main regions. Because data on Brazilian women with polycystic ovary syndrome (PCOS) are still scarce, a nation-wide collaborative study was designed to determine the prevalence of metabolic and reproductive abnormalities and the presence of anxiety and depression in Brazilian women with PCOS. In addition, the study aims at describing how these characteristics are distributed across PCOS phenotypes and at detecting associations with regional demographic and lifestyle aspects, genetic variants, and epigenetic markers.

Methods and analysis The Brazilian PCOS study is being conducted in the outpatient clinics of eight university hospitals within the public healthcare network (Unified Health System) across the country. Additional centres will be included following completion of the research ethics approval process. The sample includes women with PCOS according to Rotterdam criteria at inclusion in the study and a control group of healthy women matched by age, socioeconomic status and geographical region. Data will be collected in each centre and incorporated into a unified cloud database. Clinical, demographic, socioeconomic, psychological, metabolic, epigenetic and genotypic variables will be evaluated. The data resulting from this study will be useful to guide specific public strategies for primary and secondary prevention of metabolic and reproductive comorbidities in the PCOS population of Brazil.

Ethics and dissemination The study protocol was approved by each local Research Ethics Committee. Written informed consent will be obtained from each participant. During data collection, analysis and publication, care will be taken to ensure confidentiality of participant information. Study results will be published in peer-reviewed journals and disseminated at international conferences. This research protocol was registered with the Research Ethics Committee of HCPA, through Plataforma Brasil.

Trial registration number CAAE 18082413.9.1001.5327

Strengths and limitations of this study

- The Brazilian polycystic ovary syndrome (PCOS) study will cover all regions of Brazil and use a broad approach to collect information on different demographic, lifestyle and health aspects.
- The prospective study design and standardised procedures allow exploration of clinical, genetic and epigenetic traits.
- A control group matched by age, geographical region and socioeconomic status will adhere to the same clinical protocol as the PCOS group.
- The clinical setting may represent a referral bias, since the prevalence of more severe cases may be higher than in population studies.
- The study population may be socioeconomically skewed given the inclusion of women strictly from the public healthcare system.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex disease with heterogeneous clinical presentation, characterised by chronic anovulation and hyperandrogenism. It is the most common endocrine condition in women of reproductive age, with prevalence ranging from 8% to 13% in that age group.^{1 2} PCOS is associated with reproductive disorders and metabolic and cardiovascular disturbances including insulin resistance, obesity, hypertension, diabetes and dyslipidaemia.³ Current evidence suggests that PCOS is a polygenic, multifactorial disorder, and that its pathogenesis and clinical presentation are influenced by both environmental risk factors and genetic susceptibility; however, a causal genetic pathway has not been identified until the present moment.⁴ Among environmental factors, lifestyle aspects such as an inappropriate diet and sedentary behaviour, which

contribute to central adiposity and metabolic comorbidities, play an important role in the pathogenesis of PCOS.³

Information regarding the prevalence and severity of PCOS is still lacking in many areas of the world, especially in low-income and middle-income regions such as Africa and South America.^{1 5} In Brazil, previous works have described the characteristics of small PCOS samples, reporting a range of metabolic, cardiovascular and inflammatory alterations.⁶⁻¹¹ However, a broad study to gather nation-wide data is still missing.

Taking into account the continental dimensions of Brazil, the miscegenation of its population and the great variability in dietary and physical activity patterns across its regions, the aim of the present study is to determine the prevalence of metabolic and reproductive abnormalities and the presence of anxiety and depression in Brazilian women with PCOS. In addition, the study aims at identifying how these characteristics are distributed across PCOS phenotypes and whether they are associated with regional demographic and lifestyle aspects, genetic variants and epigenetic markers. Our hypothesis is that the prevalence of comorbidities and PCOS phenotypes may vary in different regions of the country, reflecting the differences in dietary patterns, ethnicity, and genetic and epigenetic background.

METHODS AND ANALYSIS

Study design

This case-control study—the Brazilian PCOS study—encompasses several Collaborating Centres across the country. The sample consists of women with PCOS and a control group of healthy participants matched by age, socioeconomic status and geographical region. The use of standardised procedures allows incorporation of the data collected in each centre into a unified database (figure 1). Participants will be recruited until 2021.

Setting

The first eight Collaborating Centres are distributed in the five Brazilian regions. The Coordinating Centre is located in the south, based at Universidade Federal do Rio Grande do Sul (Porto Alegre, South Region). Collaborating Centres include the following: in the southeast, Universidade Federal de Minas Gerais, Faculdade de Medicina, Universidade de São Paulo (USP), Faculdade de Medicina de Ribeirão Preto, USP and Instituto Estadual de Diabetes e Endocrinologia in Rio de Janeiro; in the north, Universidade Federal de Rondônia; in the northeast, Universidade Federal do Rio Grande do Norte; and in the midwest, Universidade Federal do Mato Grosso. Additional centres may join the study if they obtain local institutional ethics committee approval.

Sample

The study sample consists of women with PCOS receiving healthcare at university hospital outpatient clinics within the public healthcare network (Unified Health System).

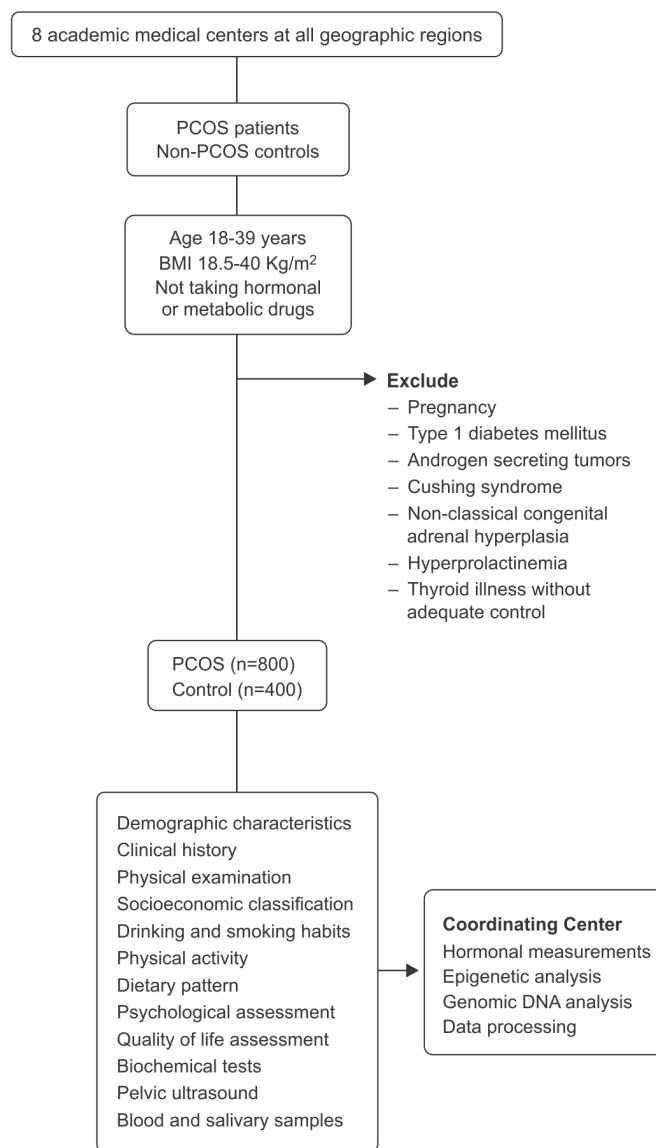


Figure 1 Flow chart of inclusion and study procedures.

PCOS is diagnosed at inclusion using the Rotterdam criteria, and participants are further classified according to PCOS phenotypes, as follows: (1) classic PCOS—women presenting oligo/amenorrhoea and/or anovulation, clinical and/or biochemical hyperandrogenism, with or without polycystic ovary morphology (PCOM) (phenotypes A and B); (2) ovulatory PCOS—having clinical and/or biochemical hyperandrogenism, PCOM and regular menstrual cycles (phenotype C) and (3) normoandrogenic PCOS—defined by oligo/amenorrhoea, PCOM, absence of clinical hyperandrogenism and normal androgen levels (phenotype D).¹²

Non-PCOS control participants are recruited in each Collaborating Centre through public advertisement or among women consulting for contraception, vaccination or reasons not related with reproductive diseases. This control group will allow detection of regional and ethnic characteristics requiring adjustment and characterisation of genotype distribution in the Brazilian population.

Eligibility criteria

Participants in the case group must meet Rotterdam PCOS criteria at enrolment. Women aged 18–39 years, recruited at least 2 years after menarche, with body mass index (BMI) varying from 18.5 to 40 kg/m², and not taking any medicine that could interfere with metabolic or hormonal assays are eligible for the study. Use of oral contraceptives must be stopped 3 months, and of metformin at least 2 months, before study enrolment. The same criteria apply to the control group.

Exclusion criteria

Type 1 diabetes, pregnancy, androgen-secreting tumours, Cushing syndrome, non-classical congenital adrenal hyperplasia, hyperprolactinaemia and thyroid illness without adequate control entail exclusion from the study. The same exclusion criteria apply to the control group.

Sample size estimation

The sample size was calculated assuming a prevalence of 60% of the classic PCOS phenotype in Latin American women (Spritzer PM, PCOS metabolic profile and phenotypes in Brazil and comparison with other Latin-American populations, 2019), with 95% CI, maximum absolute error of 5% and a design effect of 2. The case–control ratio was defined as 2:1. Based on these parameters, the sample size was estimated as 800 PCOS cases and 400 controls. In addition, to ensure that the geographical distribution of the sample reflects the population of the five Brazilian regions, the following stratification was used: south (13.7%)—110 PCOS and 55 control participants; southeast (41.5%)—332 PCOS and 167 control participants; northeast (28.4%)—227 PCOS and 113 control participants; north (8.6%)—69 PCOS and 34 control participants; and midwest (7.8%)—62 PCOS and 31 control participants.

Ethics and dissemination

During data collection, care is taken to ensure confidentiality of participants' information. Interim analyses and the full final data set will be reported in scientific publications and at research conferences.

Patient and public involvement

There is no patient and public involvement in this study.

Prospective database

Standardised questionnaires are used to collect information on demographic characteristics (age and self-reported skin colour), education, medical, family, reproductive history, lifestyle/behavioural factors, quality of life, medication/supplement inventory, alcohol consumption, cigarette smoking, dietary pattern, presence of asthma or migraine, comorbidities, menstrual pattern, presence of acne and/or hirsutism and obesity or overweight. Participants are interviewed and examinations are performed at outpatient clinics in each centre (table 1). All participants undergo a physical examination performed by a trained physician. The data are recorded in web-based

forms. Data storage and management are centralised at the Coordinating Centre using a cloud service. Importantly, all data are immediately available after collection.

Education

Educational attainment is assessed through the number of years of successful formal education, described as years at school.

Socioeconomic status

Socioeconomic status is assessed using an instrument developed by the Brazilian Association of Market Research Institutes (Associação Brasileira de Institutos de Pesquisa de Mercado). Participants are classified into five socioeconomic groups: A, B, C, D and E, with A representing the highest socioeconomic level. This classification is based on the responses to 15 questions that refer to variables such as educational attainment and items of home comfort.¹³

Alcohol intake

Alcohol consumption is self-reported: non-drinker (no drinking in the past year) or drinker. Participants are asked what type of alcohol (wine, beer, spirits, vodka, etc) they consume, the amount, and the frequency of consumption. After this detailed survey, the mean amount of alcohol (expressed as grams of alcohol consumed in a day) is estimated. Participants are then categorised as follows: low drinking (less than one drink per day), moderate drinking (less than two drinks per day), heavy drinking (more than seven drinks per week or three drinks per occasion) and binge drinking (four or more drinks on one drinking occasion).¹⁴

Smoking

For smokers, the number of cigarettes/day is registered. Former smokers are defined as individuals who report having quit smoking; they are also asked how old they were when they quit smoking. No minimum time since smoking cessation is employed in the definition of former smokers.^{15 16}

Physical activity

Participants are asked about the type and frequency of the activity performed. The questions inquire about walking or riding a bicycle for commuting and about aerobic or anaerobic activities done for fitness. Specifically, participants are asked about physical training (walking, jogging, cycling, playing volleyball, basketball, handball, soccer, swimming, dancing and gym training). Besides their current habits, participants are surveyed about their physical activity in two past periods: from 12 to 18 years of age, and from 19 years until 1 year before study enrolment. For a given activity to be considered relevant, it must be performed for at least 10 uninterrupted minutes. Physical activity is classified according to its intensity, frequency and duration using the International Physical Activity Questionnaire.^{17 18}

Table 1 Study outcomes, measurement instruments and assessment points

Outcome	Measurement instrument and assessment points
Anthropometric measures	Standardised, prospective measurements performed by trained personnel with the same calibrated equipment; weight, height, BMI, waist and hip circumference
Blood pressure/hypertension	Two measurements using the same calibrated mercury manometer showing systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 80 mm Hg; medication use
Menstrual regularity	Structured interview and classification according to International Federation of Gynaecology and Obstetrics nomenclature
Hirsutism	Modified Ferriman–Gallwey scale administered by trained physician
Socioeconomic status	Brazilian Association of Market Research Institutes questionnaire
Physical activity	Short International Physical Activity Questionnaire
Dietary pattern	Interview about frequency and amount of fruits and vegetables consumed daily and weekly
Psychological disorders	Modified Self-Reporting Questionnaire 20
Quality of life	WHO Quality of Life–BREF instrument
Dyslipidaemia	History and blood tests—total cholesterol, HDL-C, LDL-C, triglycerides—medication use
Diabetes	History and blood tests—fasting glucose—medication use
Metabolic syndrome	Joint interim statement of the International Diabetes Federation; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity; waist circumference, fast glucose, HDL-C, triglycerides and blood pressure levels
Polycystic ovarian morphology	Pelvic ultrasound with transvaginal probe whenever possible, any ovarian volume ≥ 10 cm ³ in the absence of ovulatory follicle or cyst
Epigenetic changes	Real-time quantitative PCR for microRNAs
Genotypic patterns	Real-time PCR with TaqMan assays

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Household activities such as cooking, dishwashing and house cleaning are also covered in this form, since they may represent a major physical activity for some women.

Dietary pattern

The dietary pattern refers to the amount of fruits and vegetables participants eat on a normal day. Participants are first asked if they eat fruits and vegetables. If an affirmative answer is provided, the amount of fruits and vegetables consumed on a day is estimated. In addition, information is recorded on the daily and weekly frequency of fruit and vegetable consumption. Eating five or more portions of fruits and vegetables per day, 5–7 days in a week, is defined as a marker of healthy eating.^{19 20}

Psychological assessment

Because patients with PCOS may have an increased propensity to psychological disorders,²¹ the modified Self-Reporting Questionnaire (SRQ-20) is used to determine the probability of psychological distress, mainly anxiety and depression. The SRQ-20 is recommended by the WHO for this purpose, and a validated Brazilian Portuguese version is available.²² Although the ideal cut-off is largely under debate, for the present sample a score >7 points in the SRQ-20 was established as the best estimate for the presence of a non-psychotic disorder.

This cut-off had 86% sensitivity and 89% specificity in a Brazilian study including a population of which 54% were women. In that study, the discriminant power of SRQ-20 for psychiatric screening was 0.9, and Cronbach's alpha was 0.86.^{23 24} Also, quality of life is assessed using the WHO Quality of Life–BREF instrument.²⁵ The same instruments will be administered to the control group.

Reproductive history and comorbidities

Women are asked about their age at menarche, the frequency and regularity of menses, use of medication to regulate menses, and the date of the last three menstrual periods. Menstrual pattern is then classified as regular, frequent, infrequent or amenorrhoea, according to the International Federation of Gynaecology and Obstetrics system.²⁶ The use of hormonal contraception or any type of hormone therapy is verified by asking participants to show the medication box or the medical prescription. The number of years of contraceptive use is also recorded. Information is collected on the use of non-hormonal contraceptive methods and the age at which the contraceptive method was started.

Obstetric history is assessed by asking about the number of pregnancies, miscarriages, term and preterm births, mode of delivery, and diagnosis of gestational diabetes

and preeclampsia in any pregnancy. Information on other former medical intercurrents in pregnancy is collected using an open question. Information is also collected about offspring gestational age, weight, and length at birth and about offspring with birth weight >4 kg.

The participants are then asked whether they take medication or have a known diagnosis of hypertension, diabetes and dyslipidaemia.

Family history

Participants are asked about diseases affecting family members. All diseases mentioned are recorded, but direct questions are asked about the following: hypertension, cardiovascular disease (defined as an acute myocardial infarction or angina pectoris), diabetes, thrombosis or pulmonary thromboembolism, infertility, hirsutism or thinning hair in women, and obesity.

Anthropometric measurements

Participants undergo a physical examination performed by a trained physician. Weight and height measurements are performed without shoes or heavy clothing. Weight is measured using a mechanical scale and height is measured using a stadiometer. Waist circumference is measured at the midpoint between the iliac crest and the last rib. Hip circumference is measured at the widest point of the relaxed abdomen. For both measures, participants are asked to stand with feet together and arms hanging at the sides. All these measures are taken twice, and a mean value is computed.

Blood pressure measurements

Standardised blood pressure measurements are performed twice, using the same calibrated mercury manometer. The mean of both assessments is used in the analysis. Hypertension is confirmed by asking about a previous diagnosis of hypertension or use of antihypertensive medication, or else identified during the physical examination in the presence of systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 80 mm Hg.^{27 28}

Hirsutism and acne

Hirsutism is assessed with the modified Ferriman-Gallwey scale. The score is registered as a continuous variable. A score of 8 points or more indicates hirsutism.²⁹ Hirsutism score is initially assigned by a trained investigator. After that, the participant provides her own self-reported score using a printed guide showing body areas. Shaved areas are tagged as 'shaved' and are taken into account in the analysis.

In the next step of the physical examination, the physician records the presence of acanthosis nigricans, galactorrhoea, reddish-purple streaks, acne and androgenetic alopecia. Acne is classified as follows: grade I (presence of non-inflammatory lesions, comedones only), grade II (presence of inflammatory lesions such as pustules and skin redness plus comedones), grade III (presence of widely distributed larger pustules) and grade IV (presence of cysts, nodular pustules and abscesses, with moderate

to severe scarring).³⁰ Alopecia is classified according to severity as type I, II or III.³¹

Blood and saliva sampling

Blood samples are collected from all participants between 08:00 and 10:00 after an overnight fast of 10–12 hours, during the early follicular phase of the menstrual cycle or at a random day in case of oligo/amenorrhoea. Blood is quickly centrifuged at 1036g for 8 min at room temperature and serum is separated and stored in aliquots at -80°C for future hormonal and epigenetic analyses. Additional drop samples are collected in FTA gene cards (Whatman FTA) and stored at room temperature for genetic analysis. These samples are regularly transferred to the Coordinating Centre in the city of Porto Alegre for processing, and will constitute a bio-repository.

Salivary samples are obtained at the mid-luteal phase of the menstrual cycle using a chewing roll and a collector tube especially designed for salivary hormonal tests (Salivette, Sarstedt, Nümbrecht, Germany). The tubes are centrifuged at 1000g for 2 min and stored at -20°C for subsequent progesterone assays.

Dyslipidaemia

History of dyslipidaemia is defined by self-reported hypercholesterolaemia and/or use of anti-cholesterol drugs. Blood tests are also performed to measure total cholesterol (TC) and fractions and triglyceride levels.

Diabetes and metabolic syndrome

Diabetes is determined by self-report, use of antidiabetic drugs or a fasting blood glucose level of 126 mg/dL or higher. Metabolic syndrome is defined as the presence of at least three of the following components: waist circumference >88 cm, high-density lipoprotein cholesterol (HDL-C) level <50 mg/dL, triglyceride (TG) level of 150 mg/dL or higher, blood pressure $\geq 130/85$ mm Hg and glucose level of 100 mg/dL or higher.³²

Pelvic ultrasound

A pelvic ultrasound image is obtained from all participants at each centre. The preferred method is the transvaginal approach, considered to have the best sensitivity and specificity for evaluation of the uterus and ovaries. The transabdominal procedure is used for participants with no previous vaginal intercourse. Ultrasound examination is performed during the follicular phase in participants with regular menses or on any day in those with oligo/amenorrhoea. Ovarian and uterine volumes and the number of antral follicles in each ovary are recorded. Ovarian volume $\geq 10\text{ cm}^3$ in at least one ovary, in the absence of corpus luteum, cyst or dominant follicle, will be considered as PCOM.³³

Assays

TC, HDL-C, TGs and fasting glucose levels will be determined by colorimetric-enzymatic methods in each centre. Low-density lipoprotein cholesterol (LDL-C) is estimated indirectly with the Friedewald formula: $(\text{LDL-C} = \text{TC}$

– (HDL-C +TG/5)). Hormone determinations will be performed at the Coordinating Centre. Steroid hormone binding globulin (SHBG), insulin and 25-hydroxyvitamin D (25(OH)D) levels will be measured by chemiluminescence (Abbott Architect, IL, USA), with sensitivity of 0.02 nmol/L, 1.0 µU/mL and 1.6 ng/mL, respectively. The intra-assay and interassay coefficients of variation (CVs) for SHBG, insulin and 25(OH)D are ≤5.2% and ≤9.5%, ≤4.2% and ≤5.2%, and ≤5.1% and ≤7.1%, respectively. Total testosterone (TT) is measured by high-performance liquid chromatography-tandem mass spectrometry, with a sensitivity of 7.0 ng/dL and intra-assay and interassay CVs of 4.5% and 4.7%, respectively. Androstenedione, dehydroepiandrosterone sulfate (DHEA-S) and 17 alpha hydroxyprogesterone (17α-OHP) are determined by radioimmunoassay (Beckman Coulter, Prague, Czech Republic) with sensitivity of 0.05 ng/mL, 12.33 ng/mL and 0.046 ng/mL, respectively. The intra-assay and inter-assay CVs for androstenedione, DHEA-S and 17α-OHP are ≤7.5% and ≤11.3%, ≤5.2% and ≤5.3%, ≤7.8% and ≤15.7%, respectively. Free androgen index is estimated by dividing TT (nmol/L) by SHBG (nmol/L) × 100. Homeostasis model assessment index is calculated by multiplying insulin (µIU/mL) by glucose (mmol/L) and dividing this product by 22.5. The lipid accumulation product index is calculated using the formula (waist (cm) – 58) × triglyceride concentration (mmol/L). Salivary progesterone and serum anti-Müllerian hormone (AMH) will be assayed after enrolment is completed.

Molecular analyses

For later epigenetic/microRNA analysis, serum samples are stored at –80°C. MicroRNA extraction will be made with a commercial kit (miRNeasy Serum/Plasma Advanced Kit, Qiagen, Venlo, The Netherlands). Real-time quantitative PCR will be performed to determine relative expression levels of a panel of microRNAs that have been previously screened and expressed differentially in PCOS women.³⁴

DNA will be extracted from blood samples in FTA gene cards according to the manufacturer's instructions. Single nucleotide polymorphisms (SNP) will be assessed by real-time PCR using allelic discrimination assays with TaqMan MGB primers and probes (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA).

Genotypic analyses will target genes related to endocrine and metabolic dysfunctions of PCOS, including androgen excess and metabolism, insulin resistance, metabolic syndrome and vitamin D metabolism, adipokines, inflammatory markers, hormone receptors, obesity and diabetes mellitus, hormone-binding proteins, lipoproteins, angiotensin, interleukins, β-adrenergic, Peroxisome proliferator-activated receptor (PPAR) family, cytochrome p450, irisin, kisspeptin, uncoupling protein family, and immunological factors.^{35 36}

Statistics

The Shapiro-Wilk normality test and descriptive statistics will be used to evaluate the distribution of data. Parametric variables will be described as mean and SD. Variables with non-parametric distribution will be log-transformed before statistical analysis and reported as geometric mean of their original units of measure. Non-parametric variables will be presented as median and IQR. Categorical data will be expressed as percentage and 95% CIs. Statistical tests will be applied according to data distribution in the different groups under comparison (eg, control vs PCOS or across PCOS categories). The preferred statistical tests will be χ^2 test for heterogeneity to compare proportions; one-way analysis of variance (ANOVA), followed by Bonferroni post-hoc tests for parametric variables or their equivalent of non-parametric statistics (ie, Kruskal-Wallis test); co-variance analysis will also be considered (eg, analysis of covariance) to examine the influence of variables such as age and BMI.

The association of demographic, anthropometric and metabolic variables with lifestyle habits and epigenetic markers will be investigated by unadjusted analyses, multivariate models as well as stratified by groups. An important focus of these analyses is the potential heterogeneity of risk relationships in different regions and PCOS categories. Therefore, interactions will be tested when appropriate. Logistic regression will be preferred to estimate the OR for the associations under the investigation.

Regarding molecular analyses, the agreement of genotype frequencies with Hardy-Weinberg equilibrium for each SNP will be analysed using the Pearson χ^2 test. Lewontin's D' statistic for linkage disequilibrium will be calculated for each pair of polymorphisms, and haplotypes will be inferred using the Phase 2.1 software, which uses Bayesian statistics. Linear trends will be tested to check for codominant effects of genotypes on scale data. The relationship between the outcome of interest and genotypes will be evaluated using ORs. All analyses will be performed in PASW Statistics for Windows, V.21.0 (SPSS, Chicago, IL, USA). All tests will be two-tailed and significance will be accepted at a p value < 0.05.

DISCUSSION

This is the first nation-wide study of PCOS in Brazil, a large country with a population of mixed ethnic background and marked variation in dietary and cultural traits. These characteristics provide a unique opportunity to investigate the association of lifestyle, environmental, genetic and epigenetic factors with the phenotypic expression of PCOS. We hypothesise that the prevalence of metabolic and reproductive features in women with PCOS may vary in different regions of the country and in different PCOS phenotypes.

The aetiology of PCOS is multifactorial, and investigators have focused on novel pathogenic mechanisms.^{33 34 35} In this sense, in the present study, we are collecting genomic DNA and serum samples for analyses of genotype and

epigenetic markers associated with PCOS and/or associated comorbidities. These analyses will be stratified according to the geographical region and demographic profile of participants, which will allow an unbiased assessment of genomic and epigenetic variations.

In addition, this study will generate data that will allow us to estimate the prevalence of obesity and unhealthy lifestyles to be compared in women of reproductive age from different regions of the country. These cardiometabolic modifiable risk factors will be evaluated in PCOS participants together with demographic, metabolic, genomic and epigenetic factors to determine how these variables cluster in a population of mixed genetic background. Another important aspect is the opportunity to investigate the psychological impact of PCOS.

To avoid methodological inconsistency in molecular analyses, we established a strict protocol of FTA gene cards, blood and saliva collection, storage and transportation to a single centre for analysis. In turn, pelvic ultrasound will be performed at each study site by a local expert using the best available local equipment. This invariably creates some inter-centre variability in terms of antral follicle count,³⁷ and may introduce detection bias in the inter-regional comparison of PCOS phenotypes. To overcome this limitation, we included measurements of ovarian volume, which produce less variability and have acceptable validity to assess ovarian polycystic morphology according to the Rotterdam criteria.^{12–33} We also plan to measure serum AMH levels, a reliable surrogate of ovarian polycystic morphology.³⁸ AMH levels will be assayed at a single laboratory using stored serum samples. In addition, measuring salivary progesterone in the luteal phase of women with regular menstrual cycles will allow us to ascertain ovulation and better characterise the control group and ovulatory PCOS phenotype C.

Another limitation of this study is the clinical setting, which may represent a referral bias, since the prevalence of more severe cases may be higher than in population studies.³⁹ In addition, women with PCOS referred to a secondary/tertiary healthcare unit, who agree to participate in a comprehensive examination, may constitute a selected population regarding both socioeconomic status and severity of symptoms. However, this case–control study will benefit from the planned complete characterisation of clinical and laboratorial features of PCOS as well as the exclusion of other hyperandrogenic conditions. Also, the comparison with a control group, which will be matched by age and geographical region and submitted to the same protocol as PCOS participants, including progesterone levels and pelvic ultrasonography, may be regarded as a strength; this will allow adjustment for regional and ethnic characteristics, if necessary, and enable characterisation of genotype distribution in the Brazilian population.

In conclusion, the Brazilian PCOS study will be useful to guide specific public strategies for primary and secondary prevention of metabolic and reproductive comorbidities in the PCOS population of Brazil. Beyond Brazil, the data

to be produced might also add information regarding the impact of environmental, epigenetic and ethnicity factors on the clinical expression of PCOS.

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Contributors PMS conceived the study. PMS, LBM, BRS and FVC contributed to the design of the study. PMS, LBM, BRS, FVC, KO, RMR, RAF, RVW, RMM, ALC and FMR contributed to data collection and/or analysis and interpretation of data. PMS, LBM, BRS and FMR drafted and critically revised the manuscript for important intellectual content. All the authors contributed to the further writing of the manuscript and approved the final version.

Funding This work is supported by the Brazilian National Institute of Hormones and Women's Health/Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq INCT), grant number 465482/2014-7 and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS INCT), grant number 17/2551-0000519-8.

Competing interests None declared.

Patient consent for publication Written informed consent is obtained from all participants.

Ethics approval The Brazilian PCOS study was approved by the Research Ethics Committees at all participating institutions.

Provenance and peer review Not commissioned; externally peer reviewed.

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