

RESEARCH

Increased risk of thyroid disease in Danish women with polycystic ovary syndrome: a cohort study

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Abstract

Aim: To investigate risk of thyroid disease in Danish women with PCOS.

Design: National register-based study on women with PCOS in Denmark. 18,476 women had a diagnosis of PCOS in the Danish National Patient Register. PCOS Odense University Hospital (PCOS OUH, $n = 1146$) was an embedded cohort of women with PCOS and clinical and biochemical examination. Three age-matched controls were included for each woman with PCOS ($n = 54,757$). The main outcome measures were thyroid disease (hypothyroidism, Graves' disease, goiter, thyroiditis) according to hospital diagnosis codes and/or inferred from filled medicine prescriptions. Associations between baseline TSH and development of cardio-metabolic disease was examined in PCOS OUH.

Results: The median (quartiles) age at inclusion was 29 (23–35) years and follow-up duration was 11.1 (6.9–16.0) years. The hazard ratio (95% CI) for thyroid disease development was 2.5 (2.3–2.7) ($P < 0.001$). The event rate of thyroid disease was 6.0 per 1000 patient-years in PCOS Denmark versus 2.4 per 1000 patient-years in controls ($P < 0.001$). Women in PCOS OUH with $TSH \geq 2.5$ mIU/L ($n = 133$) had higher BMI (median 29 vs 27 kg/m²), wider waist, higher triglycerides and free testosterone by the time of PCOS diagnosis compared to women in PCOS OUH with $TSH < 2.5$ mIU/L ($n = 588$). Baseline TSH did not predict later development of cardio-metabolic diseases in PCOS OUH.

Conclusions: The event rate of thyroid disease was significantly and substantially higher in women with PCOS compared to controls.

Key Words

- ▶ national
- ▶ Danish
- ▶ polycystic ovary syndrome
- ▶ register based
- ▶ ICD-10
- ▶ thyroid disease
- ▶ medicine prescriptions

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Introduction

Polycystic ovary syndrome (PCOS) comprises irregular ovulation, hyperandrogenism, and/or polycystic ovaries when no other etiology can be found (1). Frank (overt) hypothyroidism is associated with insulin resistance, dyslipidemia, weight gain, decreased levels of SHBG, anovulatory cycles, and infertility (2) and frank hypothyroidism therefore in several ways resemble a

PCOS phenotype (2). Screening for thyroid disorders is part of the recommended baseline screening of women referred with infertility, irregular menses and/or suspicion of PCOS (3).

Insulin resistance is part of the pathogenesis of PCOS (4). Insulin resistance increases the risk of metabolic syndrome (5, 6) and low-grade inflammation (4).

Low-grade inflammation and unbalanced estrogen/progesterone secretion in PCOS could have adverse effects on autoimmune function (7, 8). In accordance, women with PCOS had higher secretion of anti-thyroid, anti-nuclear (ANA), anti-ovarian, and anti-islet cell antibodies (8). A recent meta-analysis reported an odds ratio (OR) of 3.3 (95% CI 2.3–4.6) for autoimmune thyroid disease in women with PCOS (9). The risk of overt hypothyroidism in PCOS is less described than the risk of autoimmune thyroiditis. In a recent population-based study, Danish women with PCOS had higher prevalence of a hospital diagnosis of hypothyroidism than controls before the diagnosis of PCOS, but our study did not include medicine prescriptions for treatment of hypothyroidism or prospective data regarding development of frank hypothyroidism (10).

Increased autoimmunity in PCOS with higher secretion of TRAb antibodies could increase the risk for Graves' disease and higher activity of the inflammatory system could increase the risk of thyroiditis. Some authors suggested that higher LH levels in PCOS could stimulate thyroid growth and increase the risk of goiter (11), but data from small clinical studies were conflicting (12, 13). In our recent register-based study, Danish women with PCOS had higher risk of Graves' disease, thyroiditis, and goiter than controls before the diagnosis of PCOS (10). We are not aware of prospective studies regarding development of thyroid disease and use of medical treatment for thyroid disease in PCOS.

The possible clinical and metabolic side effects of subclinical hypothyroidism in PCOS are currently debated. A recent meta-analysis included TSH data from 577 women with PCOS and subclinical hypothyroidism (elevated TSH and normal thyroid hormones) and 2077 women with PCOS and normal thyroid function (14). In PCOS, subclinical hypothyroidism was linked to a more disadvantageous lipid profile and higher HOMA-ir, but glucose levels during 2-h OGTT were comparable (14). The authors requested studies regarding long-term morbidity in women with PCOS and subclinical hypothyroidism (14). It remains to be determined whether women with high-normal TSH levels represent a high-risk cardio-metabolic phenotype of women with PCOS.

Our objective was to use national registers to investigate the risk of developing thyroid disease in a Danish study population of women with PCOS. Furthermore, we investigated possible associations between TSH at diagnosis of PCOS and later development of cardio-metabolic disease in a well-described subgroup of women with PCOS.

Material and methods

We have recently reported the present study design and baseline data in detail (10). Shortly, an observational register-based cohort was drawn from Danish national health registers. The study cohort consisted of two patient populations with PCOS and one control population (Fig. 1). The cohort PCOS Denmark was formed by including all women in Denmark aged 12–60 years with a hospital diagnosis of PCOS (E282) and/or hirsutism (L680) between January 1, 1995, and the end of 2012. In addition, we included a sub-cohort of women with PCOS and/or hirsutism treated at OUH (PCOS OUH cohort). Women in PCOS OUH had available clinical and biochemical information and PCOS OUH was an embedded sub-cohort of PCOS Denmark.

The control population included three randomly drawn women from the civil population register for each patient in PCOS Denmark. Controls were assigned the index date (date of first PCOS diagnosis) of their matched PCOS case and had to be alive on this date. We have recently published data regarding prospective development of type 2 diabetes, incident fractures, and cardiovascular disease (15, 16, 17) in the study cohort.

The authors followed all of the STROBE guidelines in manuscript preparation.

Assays in PCOS OUH

Blood samples were analyzed at Odense University hospital. We have published details regarding assays recently (10, 16). Serum total testosterone and SHBG were analyzed using a specific RIA after extraction as previously described (18). This method shows close correlation with the determination of testosterone levels by mass spectrometry. Performing an oral glucose tolerance test (OGTT) was part of the routine evaluation program at OUH for newly referred women with PCOS during 1997–2003 (19).

Until 2006, TSH concentrations were measured on AutoDELTA equipment (Wallac, Turku, Finland) with three mouse anti-human monoclonal antibodies and detection by Europium as a fluorescence enhancer. From 2006, measurements were performed on an Immulite 2000 (Siemens) with dedicated reagents. Compatibility between the two analyses was assured by method comparison performed in 120 patient samples showing a regression coefficient of 0.991 and comparable means (range 0.008–49 mIU/L).

HbA1c was measured by high-performance liquid chromatography as fraction of total haemoglobin A0 using

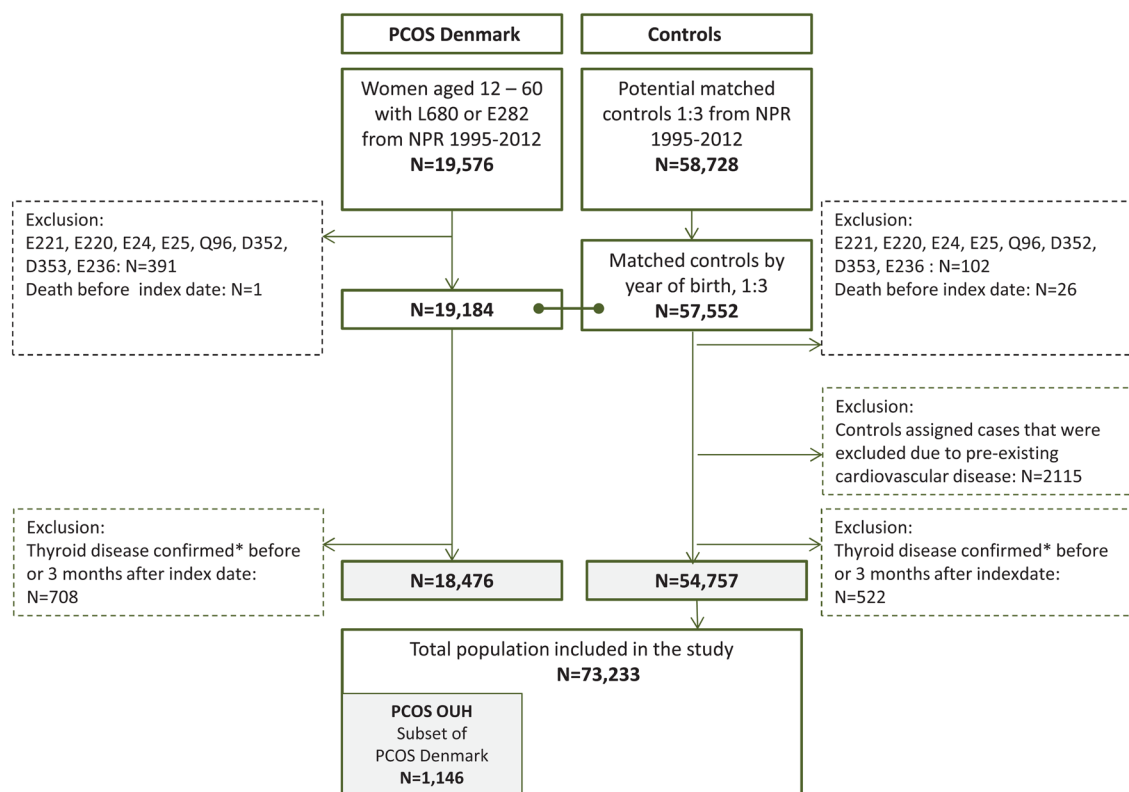


Figure 1

Flowchart of included women in PCOS Denmark and controls. Thyroid disease was defined as: 1, the presence of a diagnosis of thyroid disease in NPR according to ICD10 (Hypothyroidism (E03), struma (E04), thyrotoxicosis (E05), thyroiditis (E06), 0905 post-partum thyroiditis, iodine deficiency related thyroid disease E01, E02); or 2, prescription of drugs for treatment of thyroid disease (H03 (H03A thyroid hormone, H03B anti-thyroid medicine)); or 3, in PCOS-OUH, the occurrence of two or more measurements of TSH above reference interval; or 4, in PCOS-OUH the occurrence of two or more measurements of TSH below reference interval.

Tosoh G8 (Medinor, Broendby, Denmark) with reagents as recommended by the supplier. The analytical CV was 0.9%. Insulin was analyzed by a time-resolved fluoroimmunoassay using a commercial kit (AutoDelfia, Wallac Oy, Turku, Finland) with an intra-assay variation 2.1–3.7% and inter-assay variation 3.4–4.0%. Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TG) were analyzed by enzymatic colorimetric reactions (Modular P, Roche), while low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. Blood glucose was measured on capillary ear blood using Hemo Cue. We calculated $HOMA-r = \text{fasting insulin} \times \text{fasting blood glucose} / 22.5$ (26), reference $\leq 15 \text{ pmol mmol/l}^2$.

The Danish Health Registries

A unique personal identification number is assigned to all Danish individuals and therefore it is possible to link data from national registers at an individual level.

In the present study, information regarding hospital contacts and filled medicine prescriptions was retrieved for all included women along with dates of death if applicable. We retrieved data from National Patient Register (NPR), the National Prescription Registry, and the National Cause of Death Register in the time period 1995 to 2015 (December 31).

Outcome

Thyroid disease was our primary study outcome. Thyroid disease was defined as at least one of the following criteria: 1, the presence of a ICD10 diagnosis of thyroid disease in NPR (hypothyroidism (E03), goiter (E04), thyrotoxicosis (E05), thyroiditis (E06), 0905 post-partum thyroiditis, iodine deficiency-related thyroid disease E01, E02); or 2, use of drugs for treatment of thyroid disease according to the National Prescription Registry (H03 (H03A thyroid hormone, H03B anti-thyroid medicine)); or 3, in PCOS OUH, the occurrence of two or more measurements

of TSH above reference interval; or 4, in PCOS OUH the occurrence of two or more measurements of TSH below reference interval.

TSH was not a primary study outcome and TSH results were not entered in the original patient database for PCOS OUH. According to Danish law, it is not allowed to review patient notes for research purposes alone without the patients' informed consent. Therefore, we could not search for TSH results in the patient journals. We were able, however, to access TSH results from the county of Funen (general practitioners, Odense University Hospital) directly from the lab system and without accessing patient notes, whereas TSH measurements from other Danish hospitals could not be accessed.

All outcome criteria were defined as occurrence three months after the index date and until 2015.

In PCOS OUH, we further defined diagnoses of type 2 diabetes (T2D) (16) and cardiovascular disease (CVD) (17) according to recent publications. In brief, T2D was defined as at least one of three criteria: 1, an ICD10 diagnosis of diabetes (E11, E14 or O24); or 2, prescription of drugs for treatment of diabetes excluding prescription of metformin (A10 excluding A10BA02); or 3, the occurrence of HbA1c ≥ 48 mmol/mol and/or BG ≥ 11.1 mmol/L (16). CVD was defined as at least one of two criteria: 1, an ICD10 diagnosis of CVD (E78, G45-G46, I10-13, I20-26, I50, I63-I66, I80-82); or 2, treatment with drugs for CVD according to the National Prescription Registry (B01, C02, C07, C08, C09, C10) (17).

Exclusion criteria

We excluded all women with the ICD10 diagnoses pituitary disease (D352 (benign pituitary tumor), E236 (other pituitary disease), and D353 (craniopharyngeoma)), E220 (acromegaly), E221 (hyperprolactinemia), E24 (Cushing's syndrome), E25 (adrenogenital syndrome), and Q96 (Turner syndrome).

Participants with thyroid disease occurring before the index date and until 3 months after the index date were excluded from the analyses.

Baseline characteristics and exposure variables

Baseline characteristics regarding medicine prescriptions (antidiabetics, oral contraceptives (OCP) and fertility treatment) were extracted from the National Prescription

Registry. The women should have at least two or more dispenses of the medicine before index date. Diagnoses of obesity, diabetes, infertility, and number of births were extracted from NPR before the index date.

We defined use of OCP and number of births as exposure variables for thyroid disease. These exposures were not restricted to happen before the index date, but were defined as ever use of OCP and total number of births during 1995–2015. We defined use of OCP (ATC codes G03AA and G03AB (combined progesterone and estrogen OCP), and G03HB01 (OCP-containing cyproterone)) as at least two dispenses according to the National Prescription Registry. Number of births (ICD-10 codes O80-O84) were categorized in 0, 1, 2, or ≥ 3 and extracted from NRP.

The Charlson Comorbidity Index includes 19 comorbid conditions (20). We calculated the Charlson Comorbidity Index from the ICD-10 operationalization by Quan *et al* (21).

Statistical analyses

We presented descriptive analyses for categorical variables as frequencies and we evaluated the difference between PCOS and controls by chi-square test. Medians (with quartiles, Q1 and Q3) were tabulated for continuous variables and nonparametric test on the equality of medians were used to test for differences between groups. We considered *P* values below 0.05 statistically significant.

Incidence rates, hazard ratios (HR), 95% confidence intervals (95% CIs) and corresponding *P* values were calculated by Cox proportional hazard models. The regression analyses with thyroid disease as the study outcome and PCOS Denmark and controls as predictors were carried out crude and adjusted for one of the exposures oral contraceptives and number of births.

The risk of thyroid disease during and after pregnancy was analyzed by logistic regression analyses. We included the first birth for all women in the PCOS and control cohorts. The risk of thyroid disease during and after pregnancy was defined as an event of thyroid disease (as defined above) in the period 9 months before and 12 months after birth.

Baseline biochemical characteristics and risk of T2D and CVD according to baseline TSH was investigated in PCOS OUH. We divided women according to TSH above/below 2.5 mIU/L and TSH above/below median (1.53 mIU/L). A TSH level below 2.5 mIU/L is generally recommended before pregnancy (22). Outcomes of T2D and CVD (defined above) should occur after the index date.

Analyses were conducted using STATA 14 (StataCorp 2015) through a remote VPN access to Statistics Denmark. Data were anonymized according to Danish law and regulations.

Ethics

The study design was an open register-based cohort study. According to Danish law, the study did not need approval from the local ethics committee or institutional review board. The study was approved by the Data Protection Agency and by Statistics Denmark, project no 704175.

Results

Figure 1 is a flow chart of included women.

Register based data did not allow discrimination between women in PCOS Denmark diagnosed by Rotterdam and NIH criteria. The Rotterdam criteria were introduced in 2003. In PCOS Denmark, 7454 women were diagnosed before 2003 and 11,022 women were diagnosed after 2003.

Baseline study characteristics of the cohort are shown in Table 1. Women in PCOS Denmark and controls had a mean age of 29 years. The prevalence of ICD10 codes and medicine prescriptions related to the metabolic syndrome

occurring before the index date was significantly higher in women in PCOS Denmark compared to controls. Women with PCOS had higher prevalence of comorbidity and infertility than controls, women with PCOS had more prescriptions of OCP and drugs for fertility treatment than controls and the number of births before the index date (22 vs 20% had ≥ 1 births) was higher. Women in PCOS Denmark were comparable to women in PCOS OUH regarding age and ICD10 diagnoses for the metabolic syndrome and comorbidity before the index date, but women in PCOS Denmark had higher average number of births, lower prevalence of infertility and less use of antidiabetic medicine compared to women in PCOS OUH.

Event rates of thyroid disease are shown in Table 2. The follow-up duration was median (Q1–Q3) 11.1 (6.9–16.0) years. The thyroid disease incidence rate was 8.3/1000 person-years (PY) in PCOS OUH, 6.0/1000 PY in PCOS Denmark, and 2.4/1000 PY in controls ($P < 0.001$ PCOS Denmark vs controls). The prescription rate of thyroid hormone (H03A) in PCOS Denmark was 3.4/1000 PY compared to 1.4/1000 PY in controls and the prescription rate of anti-thyroid medication was 1.0/1000 PY in PCOS Denmark compared to 0.5/1000 PY in controls (both $P < 0.001$ PCOS Denmark vs controls).

Characteristics according to development of thyroid disease in PCOS Denmark and controls (Supplementary Table 1, see section on supplementary data given at the end of this article). Thyroid disease was diagnosed at a

Table 1 Baseline characteristics in women with PCOS and controls ($n = 73,233$).

		PCOS OUH ($n = 11,46$)	PCOS Denmark ($n = 18,476$)	Controls ($n = 54,757$)	P^a	P^b
Age (years) at PCOS diagnosis, median (Q1–Q3)		29 (22–35)	29 (24–36)	29 (24–36)	0.09	0.51
Min–max		12–58	12–60	12–60		
ICD10 codes present before index date						
Obesity	E66	154 (13)	2202 (12)	721 (1)	0.10	<0.001
Diabetes	E10,11,13,14, O24	26 (2)	403 (2)	386 (1)	0.83	<0.001
Infertility	N97, Z350	134 (12)	3365 (18)	670 (1)	<0.001	<0.001
Number of births	0	893 (78)	14,935 (80)	47,758 (87)	0.06	<0.001
	1	161 (14)	2309 (6)	4105 (8)		
	2	78 (7)	1019 (6)	2373 (4)		
	≥ 3	14 (1)	213 (1)	521 (1)		
Comorbidity		80 (7)	1540 (8)	2711 (5)	0.09	<0.001
Medicine prescriptions filled before index date						
Antidiabetics	A10	66 (6)	1627 (9)	471 (1)	<0.001	<0.001
Oral contraceptives	G03AA, G03AB, G03HB01	484 (42)	8322 (45)	14,936 (27)	0.05	<0.001
Fertility treatment	G03GA, G03GB, N04BC	115 (10)	2662 (14)	799 (1)	<0.001	<0.001

Characteristics in study cohorts at the index date.

Comorbidity was defined as a Charlson index ≥ 1 .

At least two medicine prescriptions should be redeemed to fulfill criteria for medical treatment.

P^a : Between PCOS OUH and the remainder of PCOS Denmark.

P^b : Between PCOS Denmark and controls.

Chi-square test (for categorical variables) and non-parametric test on the equality of medians (for continuous variables).

Table 2 Risk of development of thyroid disease after the index date in PCOS OUH, PCOS Denmark and controls.

	PCOS OUH (n = 1146)		PCOS Denmark (n = 18,476)		Controls (n = 54,757)		P ^a
	n (%)	Incidence rate per 1000 PY	n (%)	Incidence rate per 1000 PY	n (%)	Incidence rate per 1000 PY	
Total event rates of thyroid disease	106 (9)	8.3	1232 (7)	6.0	1521 (3)	2.4	<0.001
ICD10 thyroid disease, total	52 (5)	4.0	612 (3)	2.9	782 (1)	1.2	<0.001
Iodine deficiency-related thyroid disease (E01)	0	n/a	5 (0.03)	0.0	7 (0.01)	0.0	0.190
Hypothyroidism (E03)	25 (2)	1.9	305 (2)	1.4	327 (1)	0.5	<0.001
Goiter (E04)	27 (2)	2.0	301 (2)	1.4	413 (1)	0.7	<0.001
Thyrotoxicosis (E05)	11 (1)	0.8	248 (1)	1.2	319 (0.6)	0.5	<0.001
Thyroiditis (E06)	16 (1)	1.2	94 (0.5)	0.4	88 (0.2)	0.1	<0.001
Postpartum thyroiditis (O905)	<3	0.1	16 (0.1)	0.1	15 (0.03)	0.0	0.001
Thyroid medication, total	45 (4)	3.4	862 (5)	4.1	1079 (2)	1.7	<0.001
H03A thyroid hormone	36 (3)	2.7	702 (4)	3.4	868 (2)	1.4	<0.001
H03B anti-thyroid medication	10 (1)	0.8	215 (1)	1.0	302 (0.6)	0.5	<0.001
TSH outside reference	54 (5)	4.2	54 (0.3)	0.3	n/a	n/a	
TSH elevated (>4)	38 (3)	2.9	38 (0.2)	0.2	n/a	n/a	
TSH suppressed (<0.4)	25 (2)	1.9	25 (0.1)	0.1	n/a	n/a	

^aChi-square test between PCOS Denmark and controls.

lower median age in PCOS Denmark vs controls (32 vs 33 years, $P < 0.001$) and 41% in PCOS Denmark compared to 31% controls were aged <30 years at diagnosis of thyroid disease ($P < 0.001$). Women in PCOS Denmark who developed thyroid disease were older compared to women in PCOS Denmark and no development of thyroid disease (32 vs 29 years, $P < 0.001$).

Women with development of thyroid disease in PCOS Denmark vs women with development of thyroid disease in controls: The prescription rate of OCP was significantly higher (65 vs 50%) and the number of women with ≥ 1 births was significantly higher (48 vs 41%) in women in PCOS Denmark and development of thyroid disease vs controls with development of thyroid disease.

Proportional hazard regression analyses are given in Table 3. The HR for development of thyroid disease was 2.5 (2.3–2.7) in PCOS Denmark vs controls. Prescription of OCP and higher number of births were independent positive predictors of thyroid disease.

The risk of thyroid disease during pregnancy and 1 year post-partum is shown in Table 3. The OR for thyroid disease in pregnancy and post-partum was 2.3 (2.0–2.8) in PCOS Denmark vs controls and the OR for thyroiditis was 2.3 (1.2–4.2) in PCOS Denmark vs controls.

Baseline clinical and biochemical data in PCOS OUH is given in Table 4). In PCOS OUH, 656 women had hyperandrogenism and 500 women fulfilled the Rotterdam criteria for PCOS. In PCOS OUH, 721 women had a baseline measurement of TSH. Women in PCOS

OUH with high baseline TSH had higher BMI, waist, triglycerides, and free testosterone than women in PCOS OUH with low TSH, whereas age and SHBG were lower in women in PCOS OUH with high baseline TSH.

Baseline TSH in PCOS OUH (Table 5) did not predict later development of T2D, CVD, or depression (Supplementary Table 2).

Discussion

In the present study, we demonstrated a higher incidence rate of thyroid disease in Danish women with PCOS compared to age-matched controls. The event rate of thyroid disease was 6.0 vs 2.4/1000 PY in women with PCOS vs controls and the OR for thyroid disease was 2.9 in women with PCOS compared to controls. The average age of the study cohort was 29 years, and 7% women with PCOS compared to 3% controls were diagnosed with thyroid disease during a median follow-up of 11.1 years. Higher risk for thyroid disease in PCOS included all of the ICD-10 diagnoses hypothyroidism, goiter, thyrotoxicosis, and thyroiditis. Furthermore, higher number of births was a positive predictor of thyroid disease. In a representative subgroup of women with PCOS from our outpatient clinic, TSH above median and TSH ≥ 2.5 mIU/L was associated with a metabolic unhealthy phenotype, but baseline TSH level did not predict development of cardio-metabolic diseases.

Table 3 Crude and adjusted hazard ratios in PCOS Denmark ($n = 18,476$) and controls ($n = 54,757$) for development of thyroid disease.

Outcome: thyroid disease	Crude HR (95% CI)	Adjusted HR HR (95% CI)	Adjusted HR HR (95% CI)
PCOS (yes/no)	2.5 (2.3–2.7) $P < 0.001$	2.0 (1.8–2.2) $P < 0.001$	2.2 (2.0, 2.4) $P < 0.001$
OCP (yes/no)		2.4 (2.2–2.7) $P < 0.001$	
Number of births = 0			1
Number of births = 1			1.9 (1.7–2.2) $P < 0.001$
Number of births = 2			2.4 (1.7–2.2) $P < 0.001$
Number of births ≥ 3			2.3 (1.8–2.8) $P < 0.001$

Predictors for development of thyroid disease in PCOS Denmark and controls.

Hazard ratios are presented for crude models and models corrected for use of oral contraceptives (OCP) and number of births

To our knowledge, this is the first nationwide study to describe prospective risk of thyroid disease in women with PCOS. The present data expand our previous data in the study cohort, where women with PCOS had higher prevalence of hospital diagnoses for all thyroid diseases (hypothyroidism, goiter, thyrotoxicosis, and thyroiditis) compared to controls before the diagnosis of PCOS (10). In the present study, we added prospective data of the study cohort and the definition of thyroid disease was expanded to include medicine prescriptions and local data regarding laboratory measurements of TSH. These data ensured the

inclusion of women with thyroid diseases treated by their general practitioner.

Our finding of increased risk of hypothyroidism according to ICD10 in PCOS is in accordance with the finding of increased risk of subclinical hypothyroidism in PCOS. A recent meta-analysis by Romitti was based on 13 smaller clinical studies, none of them prospective, and included a total of 1210 women diagnosed with PCOS and 987 healthy controls (9). Four of the included studies were conducted in European study populations (23, 24, 25, 26). Anti-thyroid antibodies include anti-thyroid peroxidase (anti-TPO), thyroglobulin antibodies (anti-Tg), and thyrotrophic receptor (TRAb) antibodies (8). In the majority of included studies, autoimmune thyroid disease was defined as positive anti-TPO and/or positive anti-Tg antibodies (9). Positive anti-thyroid antibodies were observed in 26.0% women with PCOS and in 9.7% controls (9). Data regarding risk of overt hypothyroidism in PCOS were contradictive as six studies reported higher TSH in women with PCOS vs. controls, and five studies reported comparable TSH levels (9). Results from the present study suggest a considerable risk of overt hypothyroidism in women with PCOS. We are not aware of previous studies regarding the risk of Graves' disease during prospective follow-up in PCOS, whereas higher prevalence of positive TRAb antibodies in PCOS has been described recently (8). Our findings suggested that the risk of Graves' disease in PCOS was more moderate than the risk of overt hypothyroidism, but still the risk of Graves' disease in PCOS was more than two times higher than

Table 4 Risk of thyroid disease during first birth and 1 year post-partum period in PCOS OUH, PCOS Denmark and controls ($n = 24,993$).

	PCOS OUH $n = 617$	PCOS Denmark $n = 9980$	Control $n = 15,013$	PCOS Denmark vs controls $n = 24,993$	PCOS OUH vs controls $n = 1604$
	n (%)	n (%)	n (%)	OR (CI 95%) Unadjusted	OR (CI 95%) Unadjusted
Total event rates of thyroid disease	36 (5.8)	366 (3.7)	241 (1.6)	2.3 (2.0; 2.8)	3.2 (1.8; 5.6)
ICD10 thyroid disease, total	20 (3.2)	249 (2.5)	173 (1.2)	2.2 (1.8; 2.7)	2.2 (1.1; 4.3)
Hypothyroidism (E03)	10 (1.6)	154 (1.5)	78 (0.5)	3.0 (2.3; 3.9)	5.4 (1.5; 19.7)
Goiter (E04)	6 (1.0)	30 (0.3)	23 (0.2)	2.0 (1.1; 3.4)	3.2 (0.8; 12.9)
Thyrotoxicosis (E05)	4 (0.7)	64 (0.6)	59 (0.4)	1.7 (1.1; 2.3)	0.9 (0.3; 3.1)
Thyroiditis (E06)	7 (1.1)	26 (0.3)	17 (0.1)	2.3 (1.2; 4.2)	n/a
Postpartum thyroiditis (O905)	3 (0.5)	16 (0.2)	18 (0.1)	1.3 (0.7; 2.6)	1.6 (0.3; 8.0)
Thyroid medication, total	23 (3.7)	307 (3.1)	191 (1.3)	2.5 (2.1; 3.0)	2.5 (1.3; 4.8)
H03A thyroid hormone	21 (3.4)	262 (2.6)	148 (1.0)	2.7 (2.2; 3.3)	3.1 (1.5; 6.5)
H03B anti-thyroid medication	3 (0.5)	56 (0.6)	51 (0.3)	1.7 (1.1; 2.4)	0.8 (0.2; 3.2)
TSH outside reference	15 (2.4)	15 (0.2)	n/a	n/a	n/a
TSH elevated (>4)	9 (1.5)	9 (0.1)	n/a	n/a	n/a
TSH suppressed (<0.4)	10 (0.1)	10 (1.6)	n/a	n/a	n/a

The first birth for all included women was included. The risk of thyroid disease during and after pregnancy was defined as an event of thyroid disease in the period 9 months before and 12 months after birth. The risk of thyroid disease during and after pregnancy was analyzed by logistic regression analyses.

Table 5 Baseline clinical and biochemical characteristics according to TSH level around the index date in PCOS OUH (*n* = 721).

Baseline characteristics	All <i>n</i> = 721	<1.53 <i>n</i> = 360	≥1.53 <i>n</i> = 361	<i>P</i> ^a	<2.5 <i>n</i> = 588	≥2.5 <i>n</i> = 133	<i>P</i> ^a
	<i>n</i> (%)	Median (Q1–Q3)	Median (Q1–Q3)		Median (Q1–Q3)	Median (Q1–Q3)	
Age (years)	721 (100)	30 (23–36)	27 (22–34)	0.04	29 (23–35)	27 (21–36)	0.36
BMI (kg/m ²)	663 (92)	26.4 (22.7–32.2)	28.4 (23.6–33.7)	0.005	27 (23–33)	29 (24–35)	0.005
Waist (cm)	513 (72)	87 (77–102)	93 (78–106)	0.029	88 (77–103)	96 (82–108)	0.001
HbA1c (mmol/mol)	447 (62)	33.3 (31.2–36.6)	34.4 (32.0–36.6)	0.37	33.3 (31.2–36.6)	34.4 (32.2–36.6)	0.26
Fasting blood glucose (mmol/L)	269 (37)	4.8 (4.4–5.1)	4.8 (4.4–5.2)	0.19	4.8 (4.4–5.1)	4.7 (4.4–5.2)	0.86
2-h blood glucose (mmol/L)	263 (37)	6.3 (5.6–7.5)	6.5 (5.8–7.9)	0.55	6.3 (5.6–7.6)	6.7 (6.0–7.5)	0.054
Fasting insulin (pmol/L)	322 (45)	53 (36–83)	58 (38–95)	0.38	55 (38–89)	59 (45–94)	0.61
HOMA-ir (pmol mmol/l ²)	301 (42)	12.2 (7.9–18.8)	12.8 (9.0–22.1)	0.39	12.3 (8.2–20.8)	12.9 (9.6–21.2)	0.32
LDL cholesterol (mmol/l)	571 (79)	2.7 (2.2–3.3)	2.7 (2.2–3.3)	0.95	2.7 (2.2–3.3)	2.7 (2.1–3.4)	0.61
HDL cholesterol (mmol/L)	572 (79)	1.4 (1.2–1.7)	1.4 (1.1–1.6)	0.16	1.4 (1.1–1.6)	1.3 (1.0–1.7)	0.24
Cholesterol (mmol/L)	579 (80)	4.7 (4.1–5.2)	4.6 (4.0–5.3)	0.32	4.6 (4.1–5.2)	4.5 (4.0–5.5)	0.75
Triglycerides (mmol/L)	571 (79)	1.0 (0.7–1.4)	1.1 (0.8–1.5)	0.19	0.99 (0.7–1.4)	1.2 (0.8–1.6)	0.005
Total testosterone (nmol/L)	539 (75)	1.7 (1.2–2.2)	1.8 (1.3–2.5)	0.37	1.7 (1.2–2.3)	1.9 (1.3–2.6)	0.19
SHBG (nmol/L)	658 (91)	45 (31–67)	38 (27–57)	0.001	42 (30–64)	38 (26–57)	0.10
Free testosterone (nmol/L)	531 (73)	0.029 (0.020–0.047)	0.035 (0.023–0.051)	0.008	0.032 (0.020–0.047)	0.038 (0.024–0.054)	0.009
eGFR (mL/min)	617 (86)	113 (101–125)	113 (99–124)	0.60	113 (100–124)	112 (99–125)	0.48
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>P</i> ^b	<i>n</i> (%)	<i>n</i> (%)	<i>P</i> ^b
BMI ≥25 kg/m ²	251 (38)	191 (58)	221 (66)	0.02	326 (60)	86 (69)	0.04
BMI <25 kg/m ²	412 (62)	138 (42)	113 (34)		213 (40)	38 (31)	
Waist ≥88 cm	278 (54)	119 (47)	159 (61)	0.002	206 (50)	72 (70)	<0.001
Waist <88 cm	235 (46)	132 (53)	103 (39)		204 (50)	31 (30)	
Triglycerides ≥1.7 mmol/L	102 (18)	45 (17)	57 (19)	0.25	79 (17)	23 (21)	0.18
Triglycerides <1.7 mmol/L	469 (82)	227 (83)	242 (81)		384 (83)	85 (79)	

^aNon-parametric test on the equality of medians. ^bChi-squared test
TSH should be measured 12 months before until 6 months after the index date.

controls. Recent guidelines regarding follow-up of women with PCOS did not give recommendations regarding thyroid disease in PCOS (27). Our findings highlight the importance of screening for thyroid disease by the time of PCOS diagnosis and during follow-up.

Around pregnancy, we found that the risk of thyroid disease was further increased in PCOS vs controls with the highest OR for hypothyroidism. Furthermore, the positive association between number of live births and risk of autoimmune hypothyroidism was even closer in women with PCOS compared to controls. We are not aware of other studies regarding the risk of thyroid disease during and after pregnancy in PCOS. In accordance with our results, Danish register-based data in women who gave birth to singleton live born children in Denmark from 1999 to 2008 described that number of previous live births was a major risk factor for the development of autoimmune overt hypothyroidism in women aged up to 55 years (28). The authors suggested that healthy pregnancies may lead to overt hypothyroidism in women already affected by autoimmunity (28), and they concluded that number of previous pregnancies should be taken into account when evaluating the risk of hypothyroidism in premenopausal women. Women with PCOS often seek medical evaluation due to infertility, and health professionals should provide

extra attention to screening for thyroid diseases in women with PCOS before and after pregnancy.

OCP is often used to treat hyperandrogenism and irregular menses in PCOS. In accordance, 72% women with PCOS vs 34% controls had prescriptions of OCP in the present study. Interestingly, our data supported that treatment with OCP was an independent risk factor for development of thyroid disease in PCOS. The present study design did not allow us to conclude regarding the possible mechanism for this association. However, use of estrogens and progestins is known to affect the immune system and increases the risk of several autoimmune diseases (29). It is therefore possible that use of OCP could increase the risk of positive thyroid antibodies, but a previous Danish study did not support this hypothesis (30). Use of OCP can induce metabolic side effects in PCOS including increased insulin levels during OGTT (31, 32), increased inflammatory activity with higher levels of C-reactive protein (33), and weight gain (34). Activation of the inflammatory system could increase the risk of autoimmune thyroiditis (35). In addition, intensified screening for thyroid disorders in women with weight gain after use of OCP could lead to a positive association between use of OCP and thyroid disease, for example, surveillance bias. Irrespective of the mechanism, our data

highlight that screening for thyroid disease is relevant by initiation and during treatment with OCP in PCOS.

The present study design also allowed us to test the impact of TSH level at baseline PCOS diagnosis on later cardio-metabolic risk. We found that women with PCOS and high/normal TSH levels had a more adverse metabolic phenotype than women with PCOS and low/normal TSH levels. In the present study, baseline TSH level did not predict later development of a range of cardio-metabolic diseases. Our findings are in close agreement with a recent meta-analysis, where Pergialiotis *et al.* included 12 studies with TSH data from 577 women with PCOS and subclinical hypothyroidism (elevated TSH and normal thyroid hormones) (14). Subclinical hypothyroidism in PCOS was associated with lower levels of HDL, higher triglycerides, and higher HOMA-IR, whereas glucose during 2-h OGTT and levels of testosterone and SHBG were comparable (14). Based on the results, the authors recommended against supplementation with thyroid hormones in women with subclinical hypothyroidism (14). However, the authors requested studies on long-term morbidity, as subclinical hypothyroidism could result in increased morbidity in PCOS (14). The results of the present study did not support that high/normal TSH is a cardio-metabolic risk marker in PCOS. It may be argued that our study cohort was too young (average age 29 years) and lean (average BMI 28 kg/m²) and/or the follow-up duration could be too short (average 11.1 years) to allow for the relevant study outcome to occur. However, we recently documented significantly increased risk of CVD also in this relatively young study cohort (17) and 24% of women in PCOS OUH that developed CVD were lean by the time of PCOS diagnosis (17). Our results did not support a major impact of baseline TSH level and later metabolic risk, but the study design allows us to re-evaluate the study cohort upon longer follow-up.

Strengths and limitations

An important strength of this study was our study design with nationwide data in combination with available clinical and biochemical data in an embedded study cohort. This design allowed us to test hypotheses that could not be evaluated in the national cohort. Some limitations may apply to the study. The PCOS diagnosis was obtained by available ICD10 codes at hospital contacts. Some women in the control group could therefore have undiagnosed PCOS, which could underestimate

the risk of thyroid disease in PCOS. Furthermore, the introduction of the Rotterdam criteria in 2003 (3) implied the inclusion of more mild phenotypes as part of the PCOS definition. A limitation of the present study is the use of different definitions of PCOS. Furthermore, the study population included a relatively young and lean Nordic study population. Surveillance bias cannot be excluded, as women with PCOS could have more encounters with health care professionals than controls, which could lead higher capture rates for thyroid diseases and overestimation of the relative risk for thyroid disease in women with PCOS. The problem of relatively higher diagnostic vigilance for thyroid disease in PCOS could be partly overcome by selecting women with many hospital contacts for other diseases as controls. This would, however, carry a risk of selecting for less healthy women as controls.

TSH was not a primary study outcome and TSH results were not entered in the original patient database for PCOS OUH. TSH measurements from other Danish hospitals could not be accessed and lack of TSH results in some patients could have affected our study outcomes.

Development of thyroid disease could be dependent of ethnicity (9). The risk of autoimmune thyroid disease was highest in women originating from the Middle East, OR=4.6 vs 3.2 in European women (9). Therefore, the present study results need to be validated in study populations consisting of other phenotypes and with higher baseline metabolic risk.

Conclusion

The risk of development of thyroid disease was significantly higher in PCOS with hypothyroidism as the most prevalent diagnosis. Our data support that screening for thyroid diseases are relevant not only at diagnosis of PCOS, but also during follow up and especially before and during pregnancy. We did not find indication that high-normal level of TSH affected development of CVD.

Availability of data and material

The PCOS Denmark dataset generated and analyzed during the current study are not publicly available due to Danish law regarding personalized data. Data regarding PCOS OUH are available from the corresponding author on reasonable request.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-19-0377>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

DG and MA: Idea, establishment of PCOS OUH cohort, writing of manuscript; KR and BA: Idea, data retrieval and statistic calculations, revision and writing of manuscript; MN: data retrieval and constructive criticism of manuscript.

References

- Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R *et al.* The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. *European Journal of Endocrinology* 2014 **171** 1–29. (<https://doi.org/10.1530/EJE-14-0253>)
- Tagliaferri V, Romualdi D, Guido M, Mancini A, De CS, Di FC, Immediata V, Di SC & Lanzone A. The link between metabolic features and TSH levels in polycystic ovary syndrome is modulated by the body weight: an euglycaemic-hyperinsulinaemic clamp study. *European Journal of Endocrinology* 2016 **175** 433–441. (<https://doi.org/10.1530/EJE-16-0358>)
- Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertility & Sterility* 2004 **81** 19–25. (<https://doi.org/10.1016/j.fertnstert.2003.10.004>)
- Glintborg D. Endocrine and metabolic characteristics in polycystic ovary syndrome. *Danish Medical Journal* 2016 **63** B5232.
- Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK & Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care* 1999 **22** 141–146. (<https://doi.org/10.2337/diacare.22.1.141>)
- Ollila ME, West S, Keinanen-Kiukaanniemi S, Jokelainen J, Auvinen J, Puukka K, Ruokonen A, Jarvelin MR, Tapanainen JS, Franks S *et al.* Overweight and obese but not normal weight women with PCOS are at increased risk of type 2 diabetes mellitus—a prospective, population-based cohort study. *Human Reproduction* 2017 **32** 423–431. (<https://doi.org/10.1093/humrep/dew329>)
- Al-Saab R & Haddad S. Detection of thyroid autoimmunity markers in euthyroid women with polycystic ovary syndrome: a case-control study from Syria. *International Journal of Endocrinology & Metabolism* 2014 **12** e17954. (<https://doi.org/10.5812/ijem.17954>)
- Mobeen H, Afzal N & Kashif M. Polycystic ovary syndrome may be an autoimmune disorder. *Scientifica* 2016 **2016** 4071735. (<https://doi.org/10.1155/2016/4071735>)
- Romitti M, Fabris VC, Ziegelmann PK, Maia AL & Spritzer PM. Association between PCOS and autoimmune thyroid disease: a systematic review and meta-analysis. *Endocrine Connections* 2018 **7** 1158–1167. (<https://doi.org/10.1530/EC-18-0309>)
- Glintborg D, Hass RK, Nybo M, Abrahamsen B & Andersen M. Morbidity and medicine prescriptions in a nationwide Danish population of patients diagnosed with polycystic ovary syndrome. *European Journal of Endocrinology* 2015 **172** 627–638. (<https://doi.org/10.1530/EJE-14-1108>)
- Cakir E, Sahin M, Cakal E, Ozbek M & Delibasi T. Medical hypothesis: can gonadotropins influence thyroid volume in women with PCOS? *Thyroid Research* 2012 **5** 17. (<https://doi.org/10.1186/1756-6614-5-17>)
- Duran C, Basaran M, Kutlu O, Kucukaydin Z, Bakdik S, Burnik FS, Aslan U, Erdem SS & Ecirli S. Frequency of nodular goiter and autoimmune thyroid disease in patients with polycystic ovary syndrome. *Endocrine* 2015 **49** 464–469. (<https://doi.org/10.1007/s12020-014-0504-7>)
- Sinha U, Sinharay K, Saha S, Longkumer TA, Baul SN & Pal SK. Thyroid disorders in polycystic ovarian syndrome subjects: a tertiary hospital based cross-sectional study from Eastern India. *Indian Journal of Endocrinology & Metabolism* 2013 **17** 304–309. (<https://doi.org/10.4103/2230-8210.109714>)
- Pergialiotis V, Konstantopoulos P, Prodromidou A, Florou V, Papanitiou N & Perrea DN. MANAGEMENT OF ENDOCRINE DISEASE: The impact of subclinical hypothyroidism on anthropometric characteristics, lipid, glucose and hormonal profile of PCOS patients: a systematic review and meta-analysis. *European Journal of Endocrinology* 2017 **176** R159–R166. (<https://doi.org/10.1530/EJE-16-0611>)
- Rubin KH, Glintborg D, Nybo M, Andersen M & Abrahamsen B. Fracture risk is decreased in women with polycystic ovary syndrome: a register-based and population-based cohort study. *Journal of Bone and Mineral Research* 2016 **31** 709–717. (<https://doi.org/10.1002/jbmr.2737>)
- Rubin KH, Glintborg D, Nybo M, Abrahamsen B & Andersen M. Development and risk factors of type 2 diabetes in a nationwide population of women with polycystic ovary syndrome. *Journal of Clinical Endocrinology & Metabolism* 2017 **102** 3848–3857. (<https://doi.org/10.1210/jc.2017-01354>)
- Glintborg D, Rubin KH, Nybo M, Abrahamsen B & Andersen M. Cardiovascular disease in a nationwide population of Danish women with polycystic ovary syndrome. *Cardiovascular Diabetology* 2018 **17** 37. (<https://doi.org/10.1186/s12933-018-0680-5>)
- Lykkesfeldt G, Bennett P, Lykkesfeldt AE, Micic S, Moller S & Svenstrup B. Abnormal androgen and oestrogen metabolism in men with steroid sulphatase deficiency and recessive X-linked ichthyosis. *Clinical Endocrinology* 1985 **23** 385–393. (<https://doi.org/10.1111/j.1365-2265.1985.tb01096.x>)
- Glintborg D, Henriksen JE, Andersen M, Hagen C, Hangaard J, Rasmussen PE, Schousboe K & Hermann AP. Prevalence of endocrine diseases and abnormal glucose tolerance tests in 340 Caucasian premenopausal women with hirsutism as the referral diagnosis. *Fertility & Sterility* 2004 **82** 1570–1579. (<https://doi.org/10.1016/j.fertnstert.2004.06.040>)
- Charlson ME, Pompei P, Ales KL & MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *Journal of Chronic Diseases* 1987 **40** 373–383. ([https://doi.org/10.1016/0021-9681\(87\)90171-8](https://doi.org/10.1016/0021-9681(87)90171-8))
- Quan H, Sundararajan V, Halfon P, Fong A, Burnand B, Luthi JC, Saunders LD, Beck CA, Feasby TE & Ghali WA. Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. *Medical Care* 2005 **43** 1130–1139. (<https://doi.org/10.1097/01.mlr.0000182534.19832.83>)
- Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R & Vaidya B. 2014 European Thyroid Association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. *European Thyroid Journal* 2014 **3** 76–94. (<https://doi.org/10.1159/000362597>)
- Petrikova J, Lazurova I, Dravecka I, Vrbikova J, Kozakova D, Figueroa J, Vaczy Z & Rosocha J. The prevalence of non organ specific and thyroid autoimmunity in patients with polycystic ovary syndrome. *Biomedical Papers* 2015 **2** 302–306. (<https://doi.org/10.5507/bp.2014.062>)
- Janssen OE, Mehlmauer N, Hahn S, Offner AH & Gartner R. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. *European Journal of Endocrinology* 2004 **150** 363–369. (<https://doi.org/10.1530/eje.0.1500363>)
- Garelli S, Masiero S, Plebani M, Chen S, Furmaniak J, Armanini D & Betterle C. High prevalence of chronic thyroiditis in patients with polycystic ovary syndrome. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 2013 **169** 248–251. (<https://doi.org/10.1016/j.ejogrb.2013.03.003>)

- 26 Mitkov M, Nyagolova P & Orbetzova M. [Thyroid stimulating hormone levels in euthyroid women WITH polycystic ovary syndrome]. *Akusherstvo i Ginekologiya* 2015 **54** 10–15.
- 27 Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ & International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Human Reproduction* 2018 **33** 1602–1618. (<https://doi.org/10.1093/humrep/dey256>)
- 28 Carle A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB & Laurberg P. Development of autoimmune overt hypothyroidism is highly associated with live births and induced abortions but only in premenopausal women. *Journal of Clinical Endocrinology & Metabolism* 2014 **99** 2241–2249. (<https://doi.org/10.1210/jc.2013-4474>)
- 29 Williams WV. Hormonal contraception and the development of autoimmunity: a review of the literature. *Linacre Quarterly* 2017 **84** 275–295. (<https://doi.org/10.1080/00243639.2017.1360065>)
- 30 Bjergved L, Carle A, Jorgensen T, Perrild H, Laurberg P, Krejbjerg A, Ovesen L, Bulow P, I, Rasmussen LB *et al.* Parity and 11-year serum thyrotropin and thyroid autoantibody change: a longitudinal population-based study. *Thyroid* 2016 **26** 203–211. (<https://doi.org/10.1089/thy.2014.0279>)
- 31 Glintborg D, Mumm H, Holst JJ & Andersen M. Effect of oral contraceptives and/or metformin on GLP-1 secretion and reactive hypoglycaemia in polycystic ovary syndrome. *Endocrine Connections* 2017 **6** 267–277. (<https://doi.org/10.1530/EC-17-0034>)
- 32 Morin-Papunen LC, Vauhkonen I, Koivunen RM, Ruokonen A, Martikainen HK & Tapanainen JS. Endocrine and metabolic effects of metformin versus ethinyl estradiol-cyproterone acetate in obese women with polycystic ovary syndrome: a randomized study. *Journal of Clinical Endocrinology & Metabolism* 2000 **85** 3161–3168. (<https://doi.org/10.1210/jcem.85.9.6792>)
- 33 de Medeiros SF, de Medeiros MAS, Santos NS, Barbosa BB & Yamamoto MMW. Combined oral contraceptive effects on low-grade chronic inflammatory mediators in women with polycystic ovary syndrome: a systematic review and meta-analysis. *International Journal of Inflammation* 2018 **2018** 9591509. (<https://doi.org/10.1155/2018/9591509>)
- 34 Glintborg D, Altinok ML, Mumm H, Hermann AP, Ravn P & Andersen M. Body composition is improved during 12 months treatment with metformin alone or combined with oral contraceptives compared to treatment with oral contraceptives in polycystic ovary syndrome. *Journal of Clinical Endocrinology & Metabolism* 2014 **99** 2584–2591. (<https://doi.org/10.1210/jc.2014-1135>)
- 35 Czarnywojtek A, Owecki M, Zgorzalewicz-Stachowiak M, Wolinski K, Szczepanek-Parulska E, Budny B, Florek E, Waligorska-Stachura J, Miechowicz I, Baczyk M *et al.* The role of serum C-reactive protein measured by high-sensitive method in thyroid disease. *Archivum Immunologiae & Therapiae Experimentalis* 2014 **62** 501–509. (<https://doi.org/10.1007/s00005-014-0282-1>)

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