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Thyroid autoimmunity, hypothyroidism and ovarian reserve: a cross-sectional study of 5000 women based on age-specific AMH values

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STUDY QUESTION: Is there any association between thyroid autoimmunity (TAI) and diminished ovarian reserve (DOR)?

SUMMARY ANSWER: TAI and hypothyroidism are not associated with low ovarian reserve.

WHAT IS KNOWN ALREADY: TAI is a common co-existent endocrinopathy in women with primary ovarian insufficiency. Several studies support a potential link between TAI and the reduction in ovarian reserve. However, robust evidence regarding its prevalence in women with DOR is lacking.

STUDY DESIGN, SIZE, DURATION: This study is a large cross-sectional analysis of retrospective data from the Centre for Reproductive Medicine/University Hospital of Brussels. Serum measurements were taken for anti-Mullerian hormone (AMH), free thyroxine (FT4), thyroid-stimulating hormone (TSH) and anti-thyroperoxidase (anti-TPO).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Among 5076 consecutive women, 4894 women had their AMH, FT4, TSH and anti-TPO levels measured on the same day. AMH levels were plotted in relation to age for the whole patients' cohort and age-specific AMH values (per year) were considered in order to categorize women according to the AMH levels of ovarian reserve. There were 3929 women who demonstrated normal reserve, 487 women who had low ovarian reserve and 478 women who demonstrated high ovarian reserve.

MAIN RESULTS AND THE ROLE OF CHANCE: Serum FT4 and TSH levels were comparable between different ovarian reserve categories ($P = 0.611$ and 0.811, respectively). No significant differences were observed in the prevalence of positive anti-TPO antibodies among women with low (12.1%), normal (10.3%) and high (9.8%) ovarian reserve ($P = 0.423$). Finally, the prevalence of overt or subclinical hypothyroidism was comparable between the groups (4.1% in low, 4.6% in normal and 3.8% in high ovarian reserve women, $P = 0.645$).

Analysis according to the exact cause of low ovarian reserve demonstrated that women with a genetic cause of low ovarian reserve had a significantly higher prevalence of overt hypothyroidism and subclinical hypothyroidism compared with women with unexplained low ovarian reserve for their age (25 versus 3.2%, $P = 0.002$ and 18.8 versus 1.6%, $P = 0.004$, respectively). On the contrary, no significant differences were observed in the prevalence of hypothyroidism between genetic causes and iatrogenic causes ($P = 0.316$) and between iatrogenic and unexplained causes ($P = 0.219$) of low ovarian reserve.

LIMITATIONS, REASONS FOR CAUTION: This is a cross-sectional analysis based on retrospective data collection. Due to the retrospective design of this study, the presence of biases related to such a study design cannot be excluded. Furthermore, this study assessed only the association of TAI, and not autoimmunity in general, with ovarian reserve.

WIDER IMPLICATIONS OF THE FINDINGS: TAI and hypothyroidism are not associated with low ovarian reserve. Future research should focus on examining underlying mechanisms, other than TAI, which may have an effect on ovarian reserve.

STUDY FUNDING/COMPETING INTERESTS: No external funding was used for this study. No conflicts of interest are declared.

Key words: thyroid / ovarian reserve / hypothyroidism / TSH / AMH

Introduction

Diminished ovarian reserve (DOR) describes women of reproductive age having regular menses but whose response to ovarian stimulation is reduced compared with women of comparable age (Practice Committee of the American Society for Reproductive Medicine, 2012). Women with DOR represent ~24% (Ubaldi *et al.*, 2005) of the infertile population and their treatment remains a challenge for clinicians. Although several factors have been identified as causes of DOR and primary ovarian insufficiency (POI), e.g. previous chemotherapy, pelvic irradiation, previous ovarian surgery, smoking and genetic causes such as FMR1 premutation and 45X aneuploidy (De Vos *et al.*, 2010), for 90% of the cases, no specific cause has yet been identified (Nelson, 2009).

Several reports have supported a potential link between thyroid autoimmunity (TAI) and POI (Novosad *et al.*, 2003; Goswami *et al.*, 2006; Gleicher *et al.*, 2009). However, there is a lack of strong evidence regarding its prevalence in women with DOR. Recently, a retrospective cohort study provided preliminary evidence that thyroid disorders may be significantly increased in women with low ovarian reserve compared with patients with other causes of infertility (Michalakis *et al.*, 2011). Although this study included a significant number of women, DOR was defined by the presence of high basal FSH (14 UI/l), low antral follicle count (AFC < 5) and a previous poor ovarian response to stimulation. Despite that all the above-mentioned variables can successfully identify women with DOR, recent research has demonstrated that AMH is by far the most reliable marker for assessment of ovarian reserve (Nardo *et al.*, 2009; La Marca *et al.*, 2010; Dewailly *et al.*, 2014), as it can successfully predict the level of ovarian response in ovarian stimulation cycles (Polyzos *et al.*, 2012, 2013a,b). In this regard, age-specific AMH levels may be a more accurate biomarker for categorizing women in different ovarian reserve categories (Kelsey *et al.*, 2011; Nelson *et al.*, 2014).

Taking into account the above-mentioned evidence, a large cross-sectional analysis was performed in order to evaluate the association between TAI and hypothyroidism (subclinical or overt) with low ovarian reserve, as expressed by low age-specific AMH values.

Materials and Methods

A cross-sectional analysis of all consecutive women attending the Centre for Reproductive Medicine of the University Hospital of Brussels, Belgium (CRG), was conducted. The study was approved by the institutional review board of our hospital (registration number 143201319347).

Patients' eligibility criteria

Eligible women were those who: (i) had their AMH values measured in the central laboratory of UZ Brussel with the same AMH assay (Immunotech Beckman Coulter AMH ELISA kit) and (ii) had their AMH, FT4, TSH and anti-TPO levels tested on the same day at the central laboratory of UZ Brussel.

Eligible were all women who attended the CRG, either due to infertility in order to undergo fertility treatment or women who attended the unit for other reasons, e.g. oocyte donors, lesbian couples seeking fertility treatment, medical or social oocyte freezing, single women using donor sperm or women undergoing preimplantation genetic diagnosis for the prevention of hereditary diseases.

Measurement of anti-Mullerian hormone

Serum anti-Mullerian hormone (AMH) levels were assessed by the use of the Immunotech (IOT) Beckmann Coulter assay.

Blood was drawn into plain serum tubes, centrifugation was performed within 1 h and the serum was separated and immediately stored at −80°C until analysis. The AMH assay demonstrated stable intra- and inter-assay coefficients of variation <9.5% and a functional sensitivity of 0.35 ng/ml.

Measurement of thyroid hormones, TSH and anti-TPO antibodies

Blood samples taken on the day of AMH measurement were also used for the measurement of TSH, anti-TPO antibodies and free thyroxine (FT4).

Thyroid hormone values were analyzed by the RIA laboratory of the University Hospital of Brussels. Serum TSH and FT4 levels were measured using a third-generation electrochemiluminescence immunoassay (Roche Diagnostics). The reference values for TSH were 0.27–4.2 mIU/l, and the reference values for FT4 were 9.3–17.0 ng/l (12–23.3 pmol/l). The coefficients of variation of TSH were 8.7% at 0.072 mIU/l, 2.6% at 2.5 mIU/l, 2.8% at 5.9 mIU/l and 2.8% at 17.4 mIU/l and for FT4, these were 3.4% at 11.8 pmol/l, 3.8% at 26.4 pmol/l and 4.4 at 41.2 pmol/l.

Finally, the quantity of thyroid peroxidase antibodies was determined with an RIA kit (BRAHMS diagnostics), with a reference range of 0–34 kIU/l.

Patients were classified as hypothyroid based on their serum TSH and FT4 levels. Subclinical hypothyroidism was defined by having an abnormal TSH (>4.2 μIU/ml) and normal FT4, whereas overt hypothyroidism was defined as an abnormal TSH (>4.2 μIU/ml) and abnormal FT4 (<9.3 ng/l). Patients who were under supplementation with L-thyroxine prior to their serum level testing owing to overt hypothyroidism in the past were classified as hypothyroid irrespective of their serum TSH and FT4 values. For these patients, serum hormonal values were not considered in the calculation of median TSH and mean FT4 levels in different ovarian reserve categories.

Definition of ovarian reserve categories

In order to provide a reliable definition for low ovarian reserve, we utilized age-specific AMH levels for the whole population included in the study.

For this reason, we created graphs in which AMH levels were plotted in relation to age for the whole cohort of patients and age-specific AMH values (per year) were considered in order to categorize women according to the level of ovarian reserve.

Thus, women were categorized in three distinct categories: (i) low ovarian reserve (women with age-specific AMH below the 10th percentile of the values), (ii) normal ovarian reserve (women with age-specific AMH between the 10th and 90th percentile of the values) and (iii) high ovarian reserve (women with age-specific AMH above the 90th percentile of the values).

Among women who demonstrated low ovarian reserve, we manually scrutinized patients' files in order to identify risk factors that could potentially compromise their ovarian reserve. In this regard, we identified women who have had an iatrogenic cause of low ovarian reserve (ovarian surgery for endometriosis or removal of other benign lesions, ovariectomy or gonadotoxic treatment, i.e. chemo- or radiotherapy) and women with a known genetic cause of low ovarian reserve (Turner syndrome, FMR1 premutations, abnormal karyotype or any unbalanced translocation).

Main outcome measures

The primary outcome was to evaluate the prevalence of hypothyroidism (overt and subclinical) and the prevalence of positive anti-TPO antibodies between women with low, normal and high age-specific ovarian reserve.

The secondary outcome was to assess whether, among women with low ovarian reserve, the prevalence of hypothyroidism and thyroid autoimmunity was significantly higher in women without a known risk factor for low ovarian reserve, compared with women in whom any risk factor could potentially compromise their ovarian reserve, e.g. genetic causes (Turner syndrome, FMR1 premutations, abnormal karyotype or any unbalanced translocation)

or iatrogenic causes (ovariectomy, ovarian surgery or gonadotoxic chemotherapy).

The overall analysis included the whole study population and the subgroup analysis included only infertile patients.

Statistical analysis

Continuous variables were compared by using the Kruskal–Wallis test, while the Mann–Whitney *U*-test with the Bonferroni correction was used for pairwise comparisons. Categorical variables were compared with the use of the χ^2 test or Fisher's exact test.

All analyses were performed in SPSS 22 statistical software. Results were considered significant when $P < 0.05$.

Results

Overall, 5076 consecutive women, attending the CRG between 2009 and 2011, were scrutinized for eligibility (Fig. 1). Among them, 4894 were considered eligible. After categorization in relation to ovarian reserve category, 3929 patients demonstrated normal reserve, 487 were women with low ovarian reserve and 478 demonstrated high reserve.

Patients' demographics

Patients' demographic characteristics are described in Table I. As shown, age and BMI did not significantly differ between the ovarian reserve groups. Among patients seeking treatment due to infertility, there was a significant difference in the indication of infertility between low, normal and high ovarian reserve, with the former being more frequent amongst women with idiopathic infertility and the latter being more frequent amongst women with anovulatory infertility.

Thyroid function in relation to ovarian reserve

Thyroid function in relation to the level of ovarian reserve is provided in Table II. The mean (SD) serum FT4 levels were comparable between groups: low ovarian reserve, 12.0 ng/l (1.8), normal reserve 12.0 ng/l (1.9) and high reserve, 12 ng/l (1.6), $P = 0.611$. Similarly, TSH levels did not demonstrate any difference in women with low [median (IQR), 1.6 mIU/l (1.1–2.2)], normal [1.5 mIU/l (1.1–2.2)] and high ovarian reserve [1.5 mIU/l (1.1–2.2)], $P = 0.811$.

There were 508 women (10.4%) who had positive anti-TPO. The percentage of patients with positive anti-TPO did not significantly differ between women with low (12.1%), normal (10.3%) or high (9.8%) ovarian reserve, $P = 0.423$. Finally, no differences were observed between groups in the prevalence of overt or subclinical hypothyroidism as defined by serum FT4 and TSH levels (4.1% in low, 4.6% in normal and 3.8% in high ovarian reserve women, $P = 0.645$).

Thyroid function according to the cause of low ovarian reserve

Among 487 women with low age-specific ovarian reserve, 48 (9.9%) had a known cause for low ovarian reserve identified during their fertility work-up (ovarian surgery, gonadotoxic therapy or a genetic cause), whereas for 90.1% of the women, no specific cause has been identified.

Analysis according to the exact cause of low ovarian reserve demonstrated that women with genetic causes had a significant higher prevalence of hypothyroidism and subclinical hypothyroidism compared with patients with unexplained low ovarian reserve for their age [25 versus 3.2%, $P = 0.002$ and 18.8 versus 1.6%, $P = 0.004$, respectively]. On the contrary, no significant differences were observed between genetic and iatrogenic causes or between iatrogenic and unknown causes of low ovarian reserve (Table III).

Overall, 16 patients were found to have a genetic cause for low ovarian reserve (7 Turner syndrome, 5 FMR1 premutation and 4 with translocations or abnormal karyotypes). Three out of seven women with Turner syndrome had abnormal thyroid tests, whereas no patient with FMR1 premutation demonstrated any thyroid abnormality.

Subgroup analysis including only infertile patients

Thyroid function and ovarian reserve

Subgroup analysis was performed in order to evaluate thyroid function and ovarian reserve only among infertile patients. In this regard, after excluding

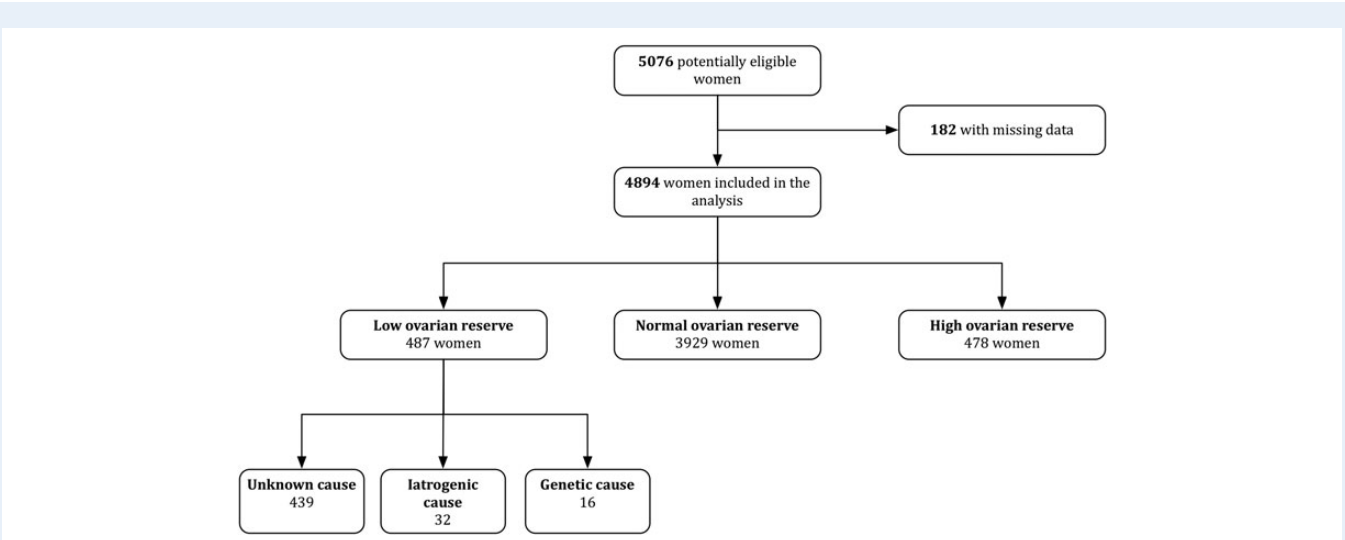

Figure 1 Flowchart of the study.

Table I Patients' baseline characteristics according to the level of ovarian reserve.

	Low ovarian reserve	Normal ovarian reserve	High ovarian reserve	*P*-value
Women (*n*)	487	3929	478	
Age (years)	32.1 (5.5)	32 (5.3)	32.1 (5.4)	0.819
BMI	24.7 (4.8)	24.5 (5.0)	24.7 (5.3)	0.442
Indication for treatment				
Infertile women	390 (80.1)	2928 (74.5)	402 (84.1)	<0.0001
Other reasons[a]	97 (19.9)	1001 (25.5)	76 (15.9)	
Reason of infertility (only infertile women included)				
Male factor	105 (26.9)	1092 (37.3)	101 (25.1)	<0.0001
Idiopathic infertility	215 (55.1)	1158 (39.5)	92 (22.9)	
Endometriosis	21 (5.4)	151 (5.2)	8 (2)	
Anovulatory	16 (4.1)	282 (9.6)	187 (46.5)	
Tubal factor	28 (7.2)	227 (7.8)	12 (3)	
Other	5 (1.3)	18 (0.6)	2 (0.5)	

[a]Oocyte donors, lesbian couples seeking fertility treatment, medical or social oocyte freezing, single women using donor sperm or women undergoing preimplantation genetic diagnosis for prevention of hereditary diseases.

Table II Thyroid function according to the level of ovarian reserve.

	Low ovarian reserve	Normal ovarian reserve	High ovarian reserve	*P*-values
Patients, *n*	487	3929	478	
Hormonal values[a]				
TSH mIU/l, median (IQR)	1.6 (1.1–2.2)	1.5 (1.1–2.2)	1.5 (1.1–2.2)	0.811
FT4 ng/l, mean (SD)	12 (1.8)	12 (1.9)	12 (1.6)	0.611
Hypothyroidism[b], *n* (%)	20 (4.1)	181 (4.6)	18 (3.8)	0.645
Subclinical hypothyroidism, *n* (%)	12 (2.5)	121 (3.1)	10 (2.1)	0.394
Positive anti-TPO[c], *n* (%)	59/486 (12.1)	403/3910 (10.3)	46/468 (9.8)	0.423

[a]Mean and medial hormonal were calculated by excluding the hormonal levels of 54 patients in total who were under L-thyroxine supplementation at the time of the serum sampling.
[b]Women with overt and subclinical hypothyroidism and also patients with hypothyroidism under treatment with levothyroxine.
[c]Thirty women with missing values.

Table III Thyroid function in women with a low ovarian reserve according to the underlying cause.

	Cause of low ovarian reserve			*P*-values
	Unknown (A)	Iatrogenic[a] (B)	Genetic (C)	
Patients, *n*	439	32	16	
Hormonal values[b]				
TSH mIU/l, median (IQR)	1.6 (1.1–2.1)	1.6 (1.0–2.4)	2.0 (1.4–3.5)	0.107
FT4 ng/l, mean (SD)	12.0 (1.8)	12.1(2.0)	11.4 (1.6)	0.620
Hypothyroidism[c,d], *n* (%)	14 (3.2)	2 (6.3)	4 (25)	<0.001
Subclinical hypothyroidism[e], *n* (%)	7 (1.6)	2 (6.3)	3 (18.8)	<0.001
Positive anti-TPO[f,g], *n* (%)	51/439 (11.6)	5/32 (15.6)	3/16 (18.8)	0.567

[a]Iatrogenic cause refers to either ovarian surgery or gonadotoxic treatment (chemotherapy or radiotherapy).
[b]Mean and medial hormonal were calculated by excluding the hormonal levels of 54 patients in total who were under L-thyroxine supplementation at the time of the serum sampling.
[c]Women with overt and subclinical hypothyroidism and also patients with hypothyroidism under treatment with levothyroxine.
[d]Pairwise comparisons: A versus B, $P = 0.2979$; A versus C, $P = 0.002$; B versus. C, $P = 0.09$.
[f]Pairwise comparisons: A versus B, $P = 0.119$; A versus C, $P = 0.004$; B versus C, $P = 0.316$.
[g]Pairwise comparisons: A versus B, $P = 0.568$; A versus C, $P = 0.421$; B versus C, $P = 1.000$.

1174 women who attended the CRG UZ Brussel with an indication for treatment other than infertility, 3720 infertile women were included. Among them, 2928 patients demonstrated normal reserve, 390 were women with low ovarian reserve and 402 demonstrated high reserve.

The mean (SD) serum FT4 levels were comparable between groups: low ovarian reserve, 11.9 ng/l (1.8), normal reserve 12.0 ng/l (1.8) and high reserve, 12.0 ng/l (1.6), $P = 0.490$. Similarly, TSH levels did not demonstrate any difference in women with low [median (IQR), 1.6 mIU/l (1.2–2.2)], normal [1.6 mIU/l (1.1–2.2)] and high ovarian reserve [1.5 mIU/l (1.1–2.4)], $P = 0.642$. TAI, assessed by positive anti-TPOs, was present in 405 women (11%). Although the percentage of patients with positive anti-TPO was higher (13.9%) in women with low ovarian reserve compared with 10.8% in normal and 8.9% in high ovarian reserve patients, the results did not differ significantly among the three groups, $P = 0.08$.

Finally, no differences were observed between groups in the prevalence of overt or subclinical hypothyroidism as defined by serum FT4 and TSH levels (4.4% in low, 4.8% in normal and 4.5% in high ovarian reserve $P = 0.894$).

Thyroid function and infertility cause

Thyroid function did not significantly differ between women with different infertility indications. The prevalence of hypothyroidism (overt or subclinical) was relatively low; 4.3% in women who planned treatment due to (their partner's) male factor infertility, 4% in unexplained infertility, 3.3% in women with endometriosis, 3.5% in women with anovulatory infertility, 1.9% in women with tubal factor and 4% when other causes were identified. Similarly, no significant differences were observed with regard to positive anti-TPO antibodies.

Discussion

To our knowledge, this is the largest study to examine the association between low ovarian reserve and TAI, including cumulatively ~5000 women. In addition, it is the first study to examine the relationship between thyroid disorders and low ovarian reserve expressed by low age-specific AMH values and not based on other variables (such as FSH levels alone, AFC or response to previous treatment) using specific thresholds which may indeed vary between different age groups.

According to our results, this cross-sectional analysis failed to demonstrate an association between TAI and ovarian reserve. TSH, FT4 and anti-TPO levels were comparable in all groups, and when the proportions of women with subclinical and overt hypothyroidism were examined, no difference was found between the different AMH groups.

When analyzing only the results obtained from women with low ovarian reserve, a significantly higher prevalence of hypothyroidism in women with a genetic cause for low ovarian reserve was observed. This finding appears to be related to the high prevalence of hypothyroidism in women with Turner syndrome, given that three out of seven women with Turner syndrome (43%) were found to have a subclinical hypothyroidism, with two of them had positive anti-TPO antibodies. On the contrary, none of the patients with either FMR1 premutations or other chromosomal abnormalities demonstrated impaired thyroid function. Although this prevalence among Turner syndrome women appears high, it is consistent with the available literature, with previous studies reporting a prevalence of 4.3–40% of hypothyroidism and 3.9–87.5% of TAI among individuals with Turner syndrome (Grossi *et al.*, 2013; Gawlik and Malecka-Tendera, 2014). Similarly, in a Japanese study including patients with Turner syndrome, the prevalence of hypothyroidism reached 31%, with about one-third being considered to have Hashimoto's thyroiditis and 70% of them receiving L-thyroxine (Fukuda *et al.*, 2009).

In addition, our study identified a higher, but non-significant, prevalence of hypothyroidism in women with iatrogenic causes of low ovarian reserve (e.g. ovarian surgery or gonadotoxic therapy) when compared with women in whom no specific cause of low ovarian reserve was identified. Although previous reports from smaller cohort studies have demonstrated that endometriosis might be linked to TAI (Gerhard *et al.*, 1991; Poppe *et al.*, 2002; Abalovich *et al.*, 2007), our analysis according to the infertility cause failed to demonstrate any significant differences. In this regard, we believe that the higher prevalence of hypothyroidism between women with low ovarian reserve who underwent ovarian surgery or chemotherapy compared with patients in whom no specific cause was identified should be mainly attributed to the small number women (only 32) in this group, and does not reflect a higher prevalence of thyroid disorders in women with endometriosis.

Our results are in contrast with a previous study showing that women with DOR had higher TSH levels (>4 mIU/ml) compared with women with normal ovarian reserve (Michalakis *et al.*, 2011) and suggesting that thyroid disorders may be related to ovarian reserve. This study along with another, which confirmed the presence of anti-thyroid antibodies in ovarian follicular fluid from women with TAI and demonstrated a very strong positive correlation between serum levels and follicular fluid autoantibodies levels (Monteleone *et al.*, 2011), led several investigators to postulate that thyroid disorders may indeed affect ovarian reserve. A proposed mechanism behind such an association could be related to the fact that anti-thyroperoxidase and anti-thyroglobulin antibodies are likely to pass through the blood–follicle barrier during the maturation period and lead to a cytotoxic environment that damages the maturing oocyte (Monteleone *et al.*, 2011).

However, despite the above-mentioned reports and the plausible scientific rationale behind such a hypothesis, our study failed to confirm these findings. A potential explanation for this discrepancy could be the number of women included. Overall, almost 5000 women were included in our study, a sample size which is actually three times the sample included in the study by Michalakis *et al.* (2011), claiming a significant association between hypothyroidism and low ovarian reserve. In addition, we included a very large group of women in whom serum AMH, FT4, TSH and anti-TPOs were measured on the same day for the whole patients' cohort. In this regard, our sample size and study design appear to be more robust compared with previous reports.

A major advantage of the current study is that we clearly defined ovarian reserve based on age-specific AMH values from the same cohort of women in order to remove the subjectivity of 'response to stimulation' and replace it with a relative stable and objective ovarian reserve parameter (AMH). This is in contrast with previous reports which defined low ovarian reserve based on other variables (such as FSH levels alone, AFC or response to previous treatment) by using specific thresholds. This may actually explain the difference observed in our study, since even though basal FSH, AFC or response to previous treatment may define women with low ovarian reserve, specific threshold values may indeed vary between different age groups and therefore one might miscategorize women with normal reserve as women with low ovarian reserve and vice versa.

Finally, in our study, we not only examined TSH levels in order to evaluate the relationship between low reserve and hypothyroidism (overt or subclinical), but we also evaluated the presence of anti-TPO antibodies in order to investigate any relationship between DOR and TAI. Thus, by failing to identify any relationship between low reserve and either hypothyroidism or thyroid autoimmunity, our results are robust enough to support the lack of any correlation between low ovarian reserve and thyroid disorders.

Although our study has several strengths, the presence of a significant limitation needs to be underlined. This is a cross-sectional analysis based on retrospective data collection. Due to the retrospective design, the presence of biases related to retrospective data collection cannot be excluded. Thus, the results should be interpreted with caution. Nonetheless, a cross-sectional analysis is considered by most as the optimal study design to assess the prevalence of conditions (such as hypothyroidism) and therefore the level of evidence provided can be considered of adequate quality.

In addition, although we defined thyroid dysfunction based on TSH and thyroid peroxidase autoantibodies (anti-TPOs), we did not evaluate thyroglobulin autoantibodies (anti-Tgs), which are also a marker of thyroid autoimmunity. This was due to the Belgian reimbursement policy which reimburses assessment of only one of the two thyroid autoantibodies. However, we must acknowledge that despite the fact that both markers are indicative of TAI, increased levels of anti-TPOs have been defined as the most sensitive marker of TAI, and high levels have been associated with thyroid dysfunction, which is not the case for anti-Tg. In this regard, given that according to the NHANES III study (1988–1994) performed in more than 13 000 individuals, anti-Tgs alone in the absence of anti-TPOs were not significantly associated with thyroid disease (Hollowell *et al.*, 2002), we believe that the omission of anti-Tg testing does not significantly bias our results.

Finally, we need to highlight the fact that our study assessed only thyroid function and TAI, and not broadly autoimmunity, in relation to age-specific ovarian reserve. Although we failed to find an association between thyroid autoimmunity and ovarian reserve, this does not mean that low ovarian reserve may not be associated with autoimmunity in general (of which TAI is just a very small spectrum). It is highly likely that there is indeed a link between autoimmunity and low ovarian reserve, given that a previous retrospective study, which defined autoimmunity broadly, including anti-phospholipid, anti-ovarian and anti-thyroid antibodies, demonstrated that women with auto-immune abnormalities demonstrate relative milder ovarian senescence (based on FSH levels) than infertile women without autoimmunity (Gleicher *et al.*, 2009). This may suggest that there is not one autoantibody, but the cumulative effect of an activated immune system, which causes inflammation and tissue destruction in different body parts, depending on the disease, or simply that there are common antigens between different organs which can be targets of autoantibodies. For example in 2–10% of the POI cases, antibodies can be directed against the steroidogenic cells in the adrenals and the ovaries as part of both the polygladular auto-immune failure type I and type II (PGAI and PGAII) (Persani *et al.*, 2009). In such cases, the incidence of POI may reach up to 60% of patients with PGA (Persani *et al.*, 2009).

In conclusion, allowing for the limitations described above, our study indicates that thyroid disorders and TAI are highly unlikely to be associated with low ovarian reserve. Future research should focus on examining underlying mechanisms, other than thyroid function, which may have an effect on ovarian reserve.

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Authors' roles

N.P.P. is responsible for the concept, design, statistical analysis and writing of the manuscript. E.S. and A.V. performed the data extraction, scrutinized patients' files and participated in writing of the manuscript. K.P., M.C. and H.T. contributed to the interpretation of the results and editing of the manuscript.

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Conflicts of interest

None declared.

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