

## Functional ovarian reserve in women with infertility and euthyroidism: what is the role of thyroid autoimmunity?

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### Abstract

**Background:** Thyroid dysfunction is the most common endocrine disorder in women of childbearing age, and is associated with menstrual irregularities, anovulation and infertility. Whether it is thyroid function, thyroid autoimmunity (AI) or both that affects functional ovarian reserve remains to be clarified. The aim of this study was to evaluate the association between functional ovarian reserve and thyroid AI in women with infertility in euthyroidism.

**Methods:** retrospective study of women with infertility, in euthyroidism, followed in a Human Reproduction Department, between May 2016 and January 2020. TSH, anti-thyroid peroxidase (TPO) antibodies, anti-thyroglobulin (TG) antibodies were measured. Functional ovarian reserve was assessed by anti-Müllerian hormone (AMH) levels with antral follicle count (AFC) performed by endovaginal ultrasound. Women with at least one of the following criteria were excluded: prior thyroidectomy, radioactive iodine treatment, cervical surgery/radiotherapy, oophorectomy, malignant/autoimmune pathology, chronic kidney disease, liver disease, polycystic ovary syndrome, current pregnancy and current medication with levothyroxine, methimazole or propylthiouracil. Results with  $p < 0.05$  were considered statistically significant.

**Results:** 730 women were evaluated, with mean age of  $34.9 \pm 3.9$  years, with positive thyroid AI ( $\geq 1$  positive antibody) present in 14.8% of cases. Anti-TPO antibodies were positive in 11.0% of patients and anti-TG antibodies in 7.0%. Mean TSH level was  $1.6 \pm 0.7$   $\mu$ IU/mL (NR: 0.4-4.0). Median body mass index (BMI) was 22.8 kg/m<sup>2</sup> (IQR 5.1). Median AMH was 1.7ng/mL (IQR 2.1), and mean AFC was  $10.2 \pm 6.3$ . Patients with positive and negative thyroid AI did not differ significantly with age ( $p = 0.133$ ), BMI ( $p = 0.784$ ), AFC ( $p = 0.508$ ) and AMH ( $p = 0.825$ ). TSH levels were significantly higher in the positive AI group ( $2.0 \pm 0.8$  vs  $1.5 \pm 0.7$   $\mu$ IU/mL;  $p < 0.001$ ).

In the univariate and multivariate analysis, only patient's age and AFC were predictive of AMH levels ( $p < 0.001$ ;  $p < 0.001$ , respectively). TSH levels, BMI and thyroid AI were not predictive of AMH levels.

In regard to AFC, in the univariate analysis, only age was predictive ( $p < 0.001$ ). TSH levels, BMI and thyroid AI were not predictive of AFC.

**Conclusions:** In this study we found that thyroid autoimmunity, in women with infertility and TSH levels in the normal range, apparently, do not have a predictive role for functional ovarian reserve.

**Keywords:** thyroid autoimmunity, euthyroidism, ovarian reserve, infertility, anti-Müllerian hormone, antral follicle count

### Background

Thyroid disease is quite prevalent in women of childbearing age and is associated with menstrual irregularities, anovulation and infertility [1, 2]. In Europe that varies between 5-7% for subclinical hypothyroidism, 0.2-4.5% for overt hypothyroidism, 0.3-1% for hyperthyroidism and 5-10% for thyroid autoimmunity [3-5].

Several studies indicate that ovarian function is adversely affected and frequency of miscarriages and infertility are increased in women with autoimmune thyroid diseases [6, 7]. These observations can be explained by the presence of thyroid hormone receptors on oocytes suggesting that thyroid hormones may influence ovarian functions [8].

The pathophysiological mechanism of the association of the thyroid AI with the ovarian reserve is not completely elucidated. It is proposed that anti-TPO antibodies pass through the blood follicle barrier during follicular development which can damage the growing follicles and oocytes [9].

The granulosa cells of small growing follicles produce AMH, and since this hormone does not significantly vary throughout the menstrual cycle, its measurement seems to be more useful in detection of ovarian reserve [10, 11]. In the majority of cases, higher AMH levels are associated with larger oocyte yields and improved pregnancy potential [12].

Whether it is thyroid function, thyroid AI or both that affects functional ovarian reserve remains to be clarified. The aim of this study was to evaluate the association between functional ovarian reserve and thyroid AI in women with infertility in euthyroidism.

## Methods

### Study population

This study investigated 730 women with infertility, in euthyroidism, followed in a Human Reproduction Department, between May 2016 and January 2020. TSH, anti-TPO antibodies, anti-TG antibodies were measured. Functional ovarian reserve was assessed by AMH levels with AFC performed by endovaginal ultrasound. Women with at least one of the following criteria were excluded: prior thyroidectomy, radioactive iodine treatment, cervical surgery/radiotherapy, oophorectomy, malignant/autoimmune pathology, chronic kidney disease, liver disease, polycystic ovary syndrome, current pregnancy and current medication with levothyroxine, methimazole or propylthiouracil.

The presented data only involved retrospective review of medical records and data retrieval from an anonymized research database.

This study was approved by the local ethics review boards (Coimbra Hospital and University Center; registration number OBS.SF.78/2021). All study methods were carried out in accordance with relevant guidelines and regulations.

### Laboratory assays

AMH, TSH and thyroid antibody status (anti-TPO and anti-TG antibodies) were measured. TSH assessments were made in blood serum, using chemiluminescence, with reference values of 0.4-4.0µIU/mL as normal range. AMH levels were assessed in blood serum by chemiluminescence assay. Thyroid autoantibodies were assessed in blood serum, utilizing chemiluminescence.

**Table 2. Differences between groups of women with positive AI vs negative AI.**

	Positive autoimmunity (n=108)	Negative autoimmunity (n=622)	p
Age (years)	35.4 ± 4.0	34.8 ± 3.8	ns
BMI (Kg/m <sup>2</sup> )	23.2 (4.7)	22.8 (5.2)	ns
AFC (n)	9.0 (6.0)	9.0 (8.0)	ns
AMH (ng/mL)	1.7 (1.9)	1.7 (2.1)	ns
TSH (µIU/mL)	2.0±0.8	1.5±0.7	<0.001
Values are presented as mean ± standard deviation; median (interquartile range).			

## Statistics

Categorical variables are presented as frequencies and percentages, and continuous variables as means and standard deviations, or medians and interquartile ranges for variables with skewed distributions. Normal distribution was checked using skewness and kurtosis. All reported p values are two-tailed, with a p value of 0.05 indicating statistical significance. The differences between groups were detected by the Student's t test for continuous variables with normal distribution and by the Mann-Whitney test and Wilcoxon test for continuous variables without normal distribution.

We used linear regression to identify the variables that made an important contribution to the variability of AMH levels and AFC, and to adjust for confounding variables.

Analyses were performed with the use of SPSS v.23.

## Results

Patient characteristics are presented in Table 1. Mean age for study participants was 34.9±3.9 years, with positive thyroid AI (≥ 1 positive antibody) present in 14.8% of cases. Anti-TPO antibodies were positive in 11.0% of patients and anti-TG antibodies in 7.0%. Mean TSH level was 1.6±0.7 µIU/mL (NR: 0.4-4.0). Median body mass index (BMI) was 22.8 kg/m<sup>2</sup> (IQR 5.1). Median AMH was 1.7ng/mL (IQR 2.1), and mean AFC was 10.2±6.3. Patients with positive and negative thyroid AI did not differ significantly in age (p=0.133), BMI (p=0.784), AFC (p=0.508) and AMH (p=0.825). TSH levels were significantly higher in the positive thyroid AI group (2.0±0.8 vs 1.5±0.7µIU/mL; p<0.001) [Table 2].

**Table 1: Descriptive analysis of 730 women.**

	n=730
Age (years)	34.9±3.9
<b>Positive autoimmunity</b> (≥ 1 positive antibody, %; n)	14.8 (108)
Anti-TPO %; n	11.0 (80)
Anti-TG %; n	7.0 (51)
TSH (µIU/mL)	1.6±0.7 (NR:0.4-4.0)
BMI (Kg/m <sup>2</sup> )	22.8 (IQR 5.1)
AMH (ng/mL)	1.7 (IQR 2.1)
AFC (n)	10.2±6.3
Values are presented as mean ± standard deviation; median (interquartile range).	

In the univariate and multivariate analysis, only patient's age and AFC were predictive of AMH levels ( $p < 0.001$ ;  $p < 0.001$ , respectively) [Table 3]. TSH levels, BMI and thyroid AI (positive vs

negative) were not predictive of AMH levels ( $p = 0.756$ ,  $p = 0.472$ ,  $p = 0.806$ , respectively).

**Table 3: Multivariate analysis – AMH.**

Independent variables	B	(95% CI)	p
Age (years)	-0.09	[0.13 - (-0.05)]	<0.001
AFC (n)	0.14	(0.11-0.16)	<0.001

The TSH cut off of  $2.5 \mu\text{IU/mL}$  was not predictive of AMH levels ( $p = 0.648$ ). The concentration of anti-TG and anti-TPO antibodies was not predictive of AMH levels. In regard to AFC, in the

univariate analysis, only age was predictive ( $p < 0.001$ ) [Table 4]. TSH levels, BMI and thyroid AI (positive vs negative) were not predictive of AFC ( $p = 0.618$ ,  $p = 0.560$ ,  $p = 0.395$ , respectively).

**Table 4: Univariate analysis – AFC.**

Independent variables	B	(95% CI)	p
Age (years)	-0.31	[-0.45 - (-0.17)]	<0.001
TSH (uIU/mL)	-0.18	(-0.92-0.55)	ns
Positive autoimmunity ( $\geq 1$ positive antibody)	-0.67	(-2.24-0.90)	ns
BMI (Kg/m <sup>2</sup> )	0.04	(-0.09-0.16)	ns

The TSH cut off of  $2.5 \mu\text{IU/mL}$  was not predictive of AFC ( $p = 0.754$ ). The concentration of anti-TG and anti-TPO antibodies was not predictive of AFC.

## Discussion

Whether it is thyroid function, thyroid AI or both that affects functional ovarian reserve has remained unresolved. The presence of anti-thyroid antibodies in ovarian follicular fluid from women with autoimmune thyroid diseases was demonstrated by Monteleone et al, and led several groups to postulate that thyroid disorders may indeed affect ovarian reserve [13].

The pathophysiological mechanism of this association could be related to the fact that anti-TPO and anti-TG 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 [13].

In this study we found that thyroid autoimmunity, in women with infertility and TSH levels in the normal range, apparently, do not have a predictive role for functional ovarian reserve. These data are supported by a publication by Osuka et al, who also reported that thyroid autoantibodies are not likely to influence ovarian reserve in euthyroid women whose TSH levels are in the normal range [14]. In a similar study, Ke et al, analyzed the association between autoimmune thyroid disease and diminished ovarian reserve and found that the presence of antibodies had no impact on ovarian reserve in euthyroid women [15].

Saglam et al, in contrast, after aged adjustment reported a strong statistical association ( $p = 0.008$ ) between autoimmune thyroid disease and AMH levels [16]. We did not find a predictive role of TSH levels in the normal range for AMH levels and AFC. These results remained overlapping when we considered the TSH cut off of  $2.5 \mu\text{IU/mL}$ .

On the other hand, Kuroda et al. reported that AMH concentrations were inversely correlated with TSH concentration (despite normal TSH concentrations) in infertile women of reproductive age [16].

The data of our study raise the possibility that thyroxin supplementation in women with TSH levels higher than  $2.5 \mu\text{IU/mL}$ , but in the normal range, prior to reproductive technique, do not increase functional ovarian reserve.

In contrast with other study [17], we did not find a significant association between BMI and AMH levels or AFC, may be because these women were in the normal weight range.

The principal reason why the presented study was limited to patients in euthyroid range without thyroxin supplementation or other thyroid medication, was, indeed, to avoid the confounding effect of this treatment in functional ovarian reserve.

In this work, we use the most reliable marker for assessment of ovarian reserve, the AMH, in contrast with previous reports which defined low ovarian reserve based on FSH levels or response to previous treatment.

The positive AI was defined based on evaluation of anti-TG and anti-TPO antibodies, in contrast with previous studies that did not evaluate anti-TG antibodies making our results more robust. There are some limitations of this study, that should be mentioned, such as the retrospective nature of the study and the data were obtained from a single center.

## Conclusions

In this study we found that thyroid autoimmunity, in women with infertility and TSH levels in the normal range, apparently, do not have a predictive role for functional ovarian reserve.

The data of our study raise the possibility that thyroxin supplementation in women with TSH levels  $> 2.5 \mu\text{IU/mL}$  and  $< 4.0 \mu\text{IU/mL}$  prior to reproductive technique, do not increase functional ovarian reserve. Further, larger prospective studies are needed to confirm these findings.

## List of abbreviations

AFC: antral follicle count  
AI: autoimmunity  
AMH: anti-Müllerian hormone  
BMI: body mass index  
TG: thyroglobulin  
TPO: thyroid peroxidase

## Declarations

### Ethics approval and consent to participate

This study was approved by the local ethics review boards (Coimbra Hospital and University Center; registration number OBS.SF.78/2021).

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