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# Assessment of different markers of ovarian reserve in women with papillary thyroid cancer treated with radioactive iodine

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## Abstract

Treatment with radioactive iodine (RAI) in women with differentiated thyroid cancer is associated with decreased serum concentrations of anti-Müllerian hormone (AMH); however, other markers have not been investigated. Therefore, this study aimed to evaluate the effect of RAI treatment on antral follicle count (AFC) and the serum concentration of inhibin B, follicle-stimulating hormone (FSH), and AMH in women with papillary thyroid cancer (PTC) treated with RAI. We examined 25 women at a median age of 33 years treated with a single dose of RAI. We divided the participants into women over ($n = 11$) and under 35 years of age ($n = 14$). Serum concentrations of inhibin B, FSH, AMH, and AFC were assessed at baseline and 1 year after RAI treatment. We found decreased AFC ($P = 0.03$), serum levels of AMH ($P < 0.01$), inhibin B ($P = 0.03$), but not FSH ($P = 0.23$), 1 year after RAI treatment in comparison to baseline in the whole group. When we compared serum levels of AMH in younger vs older women separately, we observed a significant reduction of this hormone's serum level after RAI treatment in both groups ($P < 0.01$; $P = 0.04$, respectively). We concluded that RAI treatment significantly impacts the functional ovarian reserve in premenopausal women with PTC.

**Key Words**

- ovarian reserve
- PTC
- RAI
- AMH

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## Introduction

One of the most common types of endocrine cancer, especially in young women, is thyroid cancer (1). More than 90% of all thyroid cancers are differentiated thyroid cancers (DTC), including papillary thyroid cancer (PTC) and follicular cancer (1). Accordingly, the survival prognosis in PTC patients is very good (2). Primary treatment of a patient with suspected PTC includes surgery, followed by radioactive iodine (RAI) in higher stages of PTC disease to ablate residual thyroid cancer or treat metastases (2). As a side effect of RAI, an impact on the functional ovarian reserve, which plays a crucial role in achieving pregnancy, has been observed (3). The assessment of the ovarian reserve can be based on different hormonal markers, dynamic tests, and ultrasonographic (USG) parameters, for example, antral follicle count (AFC). Markers such as the serum levels of anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), or inhibin B are relatively good prognostic markers for fertility and reliable markers for the assessment of the functional ovarian reserve (4, 5, 6).

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AMH is a glycoprotein secreted by granulosa cells of the pre-antral and early antral ovarian follicles. It is involved in regulating follicle growth, inhibiting primordial follicle recruitment, inhibiting aromatase expression, and decreasing granulosa cell sensitivity to FSH (7). A current study evaluating serum levels of AMH in women with DTC treated with RAI has revealed decreased serum concentrations of AMH during the first year after initial RAI therapy (3). Only one study has shown no changes in the serum levels of FSH in women with DTC treated with RAI (3).

No available reports evaluate other markers of the ovarian reserve, such as inhibin B and AFC, in women with DTC undergoing RAI therapy. Inhibin B is a glycoprotein hormone produced by granulosa cells in the ovary. Inhibin B is an important physiological hormone in selecting the dominant follicle during the follicular phase of the natural menstrual cycle and is involved in feedback inhibition of FSH but does not affect luteinizing hormone (LH) production (8, 9). As mentioned previously, there are no data focused on AFC before and after treatment in women with PTC with RAI.

Therefore, considering the above-mentioned data, we investigated the effect of RAI on serum concentration of inhibin B, FSH, and AMH, as well as AFC in gynecological USG in premenopausal women with PTC.

## Materials and methods

### Subjects

The Institutional Review Board approved this study (Ethics Committee of the Medical University of Białystok, Poland; approval no. R-I-002/413/2018), which complied with the principles of the Declaration of Helsinki and relevant guidelines and regulations. All subjects gave their informed consent for inclusion before they participated in the study. All the enrolled patients participated in the research voluntarily and freely.

A prospective study was conducted between November 2018 and November 2020 at the Department of Endocrinology, Diabetology, and Internal Medicine. We examined 25 women with PTC with a median age of 33 (interquartile range: 31–38). Each subject provided a full obstetric history and menstrual cycle details before and after RAI treatment. The definition of menstrual regularity was based on the 2018 International Federation of Gynecology and Obstetrics systems for nomenclature of symptoms of normal and abnormal uterine bleeding as a variation of 7–9 days, depending upon age (18–25 years for ≤9 days; 26–41 years for ≤7 days; 42–45 years for ≤9 days) and has been present for the majority of the preceding 6 months (10). Total thyroidectomy was performed in all women from 1 to 3 months before their recruitment to the study. All women were treated with a single dose of RAI (100 mCi) for advanced PTC within 3 months after operation (from 30 to 90 days). All women were treated with levothyroxine immediately after surgery (dose 100–150 µg/day). Treatment with RAI was performed in all subjects after recombinant human thyrotropin stimulation. All the patients participating in the study were Caucasians. The exclusion criteria were infertility (one woman, excluded for having undergone surgical removal of the ovaries indicated for ovarian cancer), polycystic ovarian syndrome (two women), pregnancy (two women), breastfeeding (one woman), use of hormonal contraception (three women), and history of previous RAI (two women), radiotherapy, or other medical treatment or disease that could decrease the ovarian reserve.

### Study protocol

The study protocol was the same for all patients. All women underwent a physical examination. All laboratory studies were performed in the morning, after an overnight fast, baseline, and 1 year after RAI treatment during the early follicular phase. The same gynecologist performed a transvaginal ultrasound with a 5–9 MHz transvaginal transducer (Voluson 730 Expert, GE Healthcare) in the early follicular phase (third to fifth day). The AFC was assessed, and ovarian volume was calculated using the simplified formula for a prolate ellipsoid (11).

### Biochemical analyses

All biochemical analyses were performed in a Clinical Research Centre at the Medical University of Białystok. Serum thyroid-stimulating hormone (TSH) concentration was measured using the immunoradiometric method (sensitivity 0.025 µIU/mL; intra-assay coefficient of variation (CV) – 0.6%; inter-assay CV – 2.1%), and serum-free T3 (fT3) (sensitivity 0.3 pg/mL; intra-assay CV – 6.4%; inter-assay CV – 5.5%), and serum-free T4 (fT4) (sensitivity 0.03 ng/dL; intra-assay CV – 10.3%, inter-assay CV –7.6%) concentrations were detected with RIA kits (all DIAsource ImmunoAssays S.A., Belgium). The levels of serum FSH, LH, and estradiol (E2) were assessed using the electrochemiluminescence method intended for use on Cobas E411 device(Cobas E411 immunoassay analyzer,

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Roche Diagnostics GmbH) (LH: intra-assay CV – below 1.8%, inter-assay CV – below 5.2%; FSH: intra-assay CV – below 2%, inter-assay CV – below 5.3%; E2: intra-assay and inter-assay CV – below 2 and 5.3%, respectively). Serum AMH concentrations were determined through enzyme immunoassay (Beckman Coulter, Marseille, France). The lowest concentration of AMH detectable with a 95% probability was 0.08 ng/mL. The intra-assay and inter-assay CV were below 5.4 and 5.6%, respectively. Inhibin B serum concentrations were measured using an ELISA kit (Cloud Clone Corp., Houston, TX, USA (Catalogue no. SEA760Ra)) characterized by lower limit of detection equal to 2.84 pg/mL; inter-assay CV < 12% and intra-assay CV < 10%.

### Statistical analysis

Statistical analyses were performed using Statistica 13.3 package (Statsoft, Cracow, Poland). The variables were tested for normality of distribution using Shapiro–Wilk test. Due to the non-normal distribution of the data, Wilcoxon and Mann–Whitney *U* tests were used to examine the statistical differences in the parameters before and after RAI treatment in women with PTC. Spearman's test was used for correlation analysis, and the ensuing results were expressed using Spearman's correlation coefficient (*r*). Continuous variables were expressed as the median and interquartile range. A *P* value <0.05 was considered statistically significant. We retrospectively performed statistical analysis for statistical power. The minimal sample size to detect significant differences at $\alpha < 0.05$ and $1 - \beta > 0.80$ was $N = 20$.

**Table 1** Stage of advancement (TNM), American Thyroid Association risk stratification system, vascular invasion, extrathyroidal extension, multifocality, and treatment response.

| **Study group** ($n = 25$) | |
|---|---|
| Stage of advancement (TNM) AJCC/TNM staging system (8th edition) | **T stage**<br>T1a, $n = 5$<br>T1b, $n = 6$<br>T2, $n = 10$<br>T3, $n = 2$<br>T4, $n = 2$<br>**N stage**<br>N0, $n = 13$<br>N1a, $n = 11$<br>N1b, $n = 1$<br>**M stage**<br>M0, $n = 25$<br>M1, $n = 0$ |
| Vascular invasion | $n = 5$ |
| Extrathyroidal extension | $n = 1$ |
| Multifocality | $n = 11$ |
| ATA Risk Stratification System (2015) (2) | Low, $n = 0$<br>Intermediate, $n = 22$<br>High, $n = 3$ |
| Recurrence after 1 year follow-up | $n = 1$ |

TNM, cancer tumor, node, metastasis classification for differentiated and anaplastic thyroid cancer (T1a, tumor size ≤ 1 cm in greatest dimension limited to the thyroid; T1b, tumor > 1 cm but ≤2 cm in greatest dimension, limited to the thyroid; T2, tumor size > 2 cm but ≤4 cm, limited to the thyroid; T3, tumor size > 4 cm, limited to the thyroid; T4, tumor with gross extrathyroidal extension; N, lymph node metastasis; M, metastasis; *n*, number of patients).

## Results

This study enrolled 25 women with classical thyroid papillary cancer. In all women, the tumor, node, metastats (TNM) stage of the disease was assessed (T1–T3, N0 or N1, M0) (Table 1). All women had menstrual regularity before RAI treatment, whereas four had menstrual irregularity 1 year after treatment (16%). None of the women became pregnant during the 1-year observation period.

We observed higher serum concentration of TSH before RAI treatment (median 0.6 (interquartile range: 0.08–3.8)) in comparison with 6 weeks after RAI treatment (median 0.12 (interquartile range: 0.03–0.3)) ($P = 0.03$) and in comparison with 1 year after RAI treatment (median 0.1 (interquartile range: 0.01–0.3)) ($P = 0.03$). We did not notice differences between serum levels of TSH 6 weeks after RAI treatment in comparison with 1 year after RAI treatment ($P = 0.5$). We did not observe significant difference in serum levels of fT4 ($P = 0.8$) and fT3 ($P = 0.9$) before RAI treatment in comparison to 1 year after RAI treatment. Additionally, we did not observe any significant differences between the serum concentrations of LH ($P = 0.9$) and estradiol ($P = 0.68$) after RAI treatment and the respective concentrations before RAI treatment (Table 2).

We found decreased serum levels of AMH ($P < 0.01$), inhibin B ($P = 0.03$), and AFC ($P = 0.03$) 1 year after RAI treatment in women with PTC relative to baseline values. Serum levels of FSH ($P = 0.23$) did not change after RAI treatment when compared to baseline values (Table 3).

Additionally, we noticed decreased levels of AMH in 72% of women after RAI treatment, and the median serum AMH level was 13% lower than that before treatment (2.3 ng/mL vs 2.0 ng/mL; $P < 0.01$). When we plotted the AMH values on percentile grids (12), we observed a decrease in percentiles in all women 1 year after RAI treatment compared to pre-treatment values.

We also divided our participants into groups of women over ($n = 11$) and under ($n = 14$) 35 years of age. As we expected, women over the age of 35 years had lower serum

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**Table 2** Clinical and biochemical characteristics of the studied groups.

| | Before RAI ($n$ = 25) | 1 year after RAI ($n$ = 25) | *P* value |
|---|---|---|---|
| Age (years) | 33 (31–38) | – | – |
| Menarche (age) | 14 (13–15) | – | – |
| TSH (uIU/mL) | 0.6 (0.08–3.8) | 0.1 (0.01–0.3) | 0.03 |
| fT4 (ng/dL) | 1.2 (1.0–1.4) | 1.3 (1.1–1.4) | 0.8 |
| fT3 (pg/mL) | 2.6 (2.4–3.0) | 2.8 (2.6–3.1) | 0.9 |
| LH (mIU/mL) | 7.3 (4.7–10.3) | 7.8 (6.1–15.3) | 0.9 |
| Estradiol (pg/mL) | 93.1 (43.3–134.4) | 84.2 (40.8–175) | 0.68 |

Values are expressed as median (interquartile range).
FSH, follicle-stimulating hormone; fT4, free T4; fT3, free T3; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

levels of AMH than younger patients before (median 1.0 ng/mL vs 2.7 ng/mL; $P=0.01$) and after RAI treatment (median 0.5 ng/mL vs 2.4 ng/mL; $P=0.01$). When we compared serum levels of AMH in younger and older women separately, we observed a significant reduction in the concentration of this hormone after RAI treatment in both groups ($P=0.04$ and $P < 0.01$, respectively). However, we did not notice significant differences in the serum levels of inhibin B, FSH, and AFC before and after RAI treatment when we analyzed women under and over 35 years old separately (all $P > 0.05$).

As expected, we observed a negative relationship between serum levels of AMH and age ($r=-0.59$, $P < 0.01$) before RAI treatment. We did not notice any significant relationships between serum levels of AMH and age at menarche, thyroid hormones, FSH, or AFC in USG before RAI treatment (all $P > 0.05$) (Table 4) and after RAI treatment (all $P > 0.05$). We found an inverse correlation between serum levels of inhibin B and fT4 ($r=-0.45$, $P=0.02$) before RAI treatment and age at menarche ($r=-0.8$, $P=0.02$) after RAI treatment. We did not notice any significant relationships between serum levels of inhibin B and fT3, FSH, or AFC in USG before and after RAI treatment (all $P > 0.05$) (Table 4).

**Table 3** Assessment of ovarian reserve markers before and after RAI ablative therapy.

| | Before RAI ($n$ =25) | One year after RAI ($n$ =25) | *P* value |
|---|---|---|---|
| AMH (ng/mL) | 2.3 (1.5–3.9) | 2.0 (0.4–3.3) | <0.01 |
| Inhibin B (pg/mL) | 14.1 (10.5–28) | 13.8 (8.2–17.8) | 0.03 |
| FSH (mIU/mL) | 5.8 (4.0–7.4) | 6.1 (4.9–8.6) | 0.23 |
| AFC (number) | 7 (5–14) | 5 (4–11) | 0.03 |

Values are expressed as median (interquartile range).
AFC, antral follicle count; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone.

## Discussion

In the present study, we observed lower serum levels of AMH and other functional ovarian reserve markers (inhibin B and AFC) 1 year after RAI treatment compared to baseline in premenopausal women with PTC. The previous studies evaluated ovarian reserve only by estimating serum levels of AMH (3, 13, 14, 15). Giusti *et al.* did not find significant differences in serum levels of AMH between women receiving RAI and control women not treated with RAI (13); however, they did not estimate the serum levels of this hormone before RAI as we did. On the contrary, Acibcucu *et al.* observed lower levels of AMH after RAI treatment vs control women who were not treated with RAI (14). However, they also did not compare serum AMH levels before and after RAI treatment. In the literature, few studies focused on serum AMH levels before and after RAI treatment in women with DTC (3, 15). A recent systemic review and meta-analysis showed a significant decrease in the serum AMH levels after RAI treatment in DTC women. Interestingly, a decrease in pregnancy rates after RAI therapy has not been observed. This could be because they carried out a retrospective study and not a prospective study (16). Therefore, prospective studies are needed to confirm their results. In another study, a profound decrease in serum AMH levels after RAI treatment, even after a small dose (30 mCi) of RAI, has been observed (15); however, this observation was not confirmed by other researchers (17). Interestingly, Evranos *et al.* measured serum AMH levels at 3, 6, and 12 months after RAI treatment but did not find any significant differences among the three timepoints (3). On the contrary, the lowest AMH levels were shown at 3 months after treatment with RAI without complete recovery at 1 year (15). Therefore, we estimated the functional ovarian reserve markers at baseline and 1 year after RAI treatment.

In our study, we observed that 72% of women had lower serum AMH concentrations 1 year after RAI treatment than they did at baseline, and the median serum AMH level was 13% lower than before treatment. These findings are similar to those of Yaish *et al.*, who found that 82% of women had final values of AMH below baseline levels, and serum AMH levels were 32% lower than before RAI treatment (15). A greater decrease in serum levels of AMH was observed by Velsen *et al.*, who reported a 50% drop in AMH levels after RAI treatment.

Kelsey *et al.* published a validated model of serum AMH concentration from conception to menopause (18). Analyses of the model show that the dynamics of circulating AMH levels throughout life can be split into

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**Table 4** The relationship between serum levels of AMH and inhibin B with age, age at menarche, and biochemical parameters before and after RAI treatment ($n$ =25).

| | Serum levels of AMH before RAI treatment | Serum levels of inhibin B before RAI treatment | Serum levels of inhibin B 1 year after RAI treatment |
|---|---|---|---|
| Age (years) | ($r$ = −0.59, $P$ < 0.01) | ($r$ = −0.19, $P$ = 0.35) | ($r$ = −0.1, $P$ = 0.6) |
| Age at menarche (age) | ($r$ = −0.54, $P$ = 0.2) | ($r$ = −0.2, $P$ = 0.8) | ($r$ =−0.8, $P$ = 0.02) |
| fT4 (ng/dL) | ($r$ = 0.05, $P$ = 0.8) | ($r$ = −0.45, $P$ = 0.02) | ($r$ = −0.35, $P$ = 0.11) |
| fT3 (pg/mL) | ($r$ = 0.4, $P$ = 0.05) | ($r$ = −0.08, $P$ = 0.7) | ($r$ = −0.2, $P$ = 0.35) |
| FSH (mIU/mL) | ($r$ = -0.5, $P$ = 0.45) | ($r$ = 0.27, $P$ = 0.18) | ($r$ = −0.06, $P$ = 0.75) |
| AFC | ($r$ = 0.4, $P$ = 0.5) | ($r$ = −0.5, $P$ = 0.39) | ($r$ = 0.8, $P$ = 0.1) |

AFC, antral follicle count; FSH, follicle-stimulating hormone; fT4, free T4; fT3, free T3; RAI, radioactive iodine.

several distinct phases. A peak shortly after birth confirms that girls also undergo a 'mini puberty' of the neonate, following which there is a sustained rise to the age of 9 years. There is an inflection with even a slight decline during the pubertal ages (9–15 years), followed by a second growth phase to a peak at an age of 25 years. After this, there is a steady decline to undetectable levels at an average age of 50–51 years, corresponding to menopause (18, 19). In a study involving 17,120 women of reproductive age (from 24 to 50 years old), the average yearly decrease in the median serum AMH value was 0.2 ng/mL/year through the age of 35 years and then diminished to 0.1 ng/mL/year after the age of 35 years (20). In our group, the median serum levels of AMH in women under 35 years of age decreased by 0.3 ng/mL/year, whereas those in women over 35 years of age decreased by 0.5 ng/mL/year. Moreover, in our group, the value for the centile of AMH (21) was also lower after RAI treatment than before RAI treatment. Therefore, as we demonstrated in our study, RAI treatment is associated with significantly decreased serum levels of AMH. Our results are similar to those obtained by previous researchers who demonstrated that RAI treatment is connected with a significant decrease in AMH concentrations in women with DTC treated with RAI (16). Interestingly, Mittica *et al.* observed a non-significant reduction in AMH on ANOVA testing in women treated with RAI and in women after thyroidectomy. However, on applying Wilcoxon's paired test, AMH significantly decreased between the first and second evaluations only in women treated with RAI, with no significant variations observed between the first and second determination in groups after thyroidectomy (22).

It has been shown that when women reach the age of 35 the follicular growth begins to accelerate, causing an increased loss of the residual follicular stock in combination with a gradual increase in circulating levels of FSH (23). The age-related decline in fecundity is indirectly associated to the follicular pool as the progressive reduction is accompanied by an associated decline in oocyte quality (24). It has been presented that serum AMH and AFC started to decline in women between 34 and 35 years old (12), and the age of 35–39 years was a predictor which significantly increased incidence of fertility treatment (HR 1.66, 95% CI 1.11–2.48, $P$=0.038) (25). Moreover, a Danish prospective study of 1338 infertile couples demonstrated an increased chance of delivery, if the woman's age was below 35 years compared to women aged 35 or older (74.9% vs 52.2%) (26). Additionally, in the study conducted on the group of women with thyroid cancer treated with RAI, Velsen *et al.* observed that women younger than 35 years of age had a gentler decrease in AMH levels (17), and the authors proposed less aggressive RAI treatment in low-risk patients, especially those over 35 years of age with the desire to have children (17). Low-risk patients have intrathyroidal DTC, with no evidence of extrathyroidal extension, vascular invasion, or metastases (2). In other study, birth rate among women aged 35–39 was significantly decreased in those who received RAI vs those who did not (27), and it has been reported earlier menopause as a result of ovarian damage from RAI in women treated at the average age of 35 years for thyroid cancer in comparison to women not treated with RAI (28). Therefore, we divided our group into women over and under 35 years of age. In our study, we observed an inverse relationship between age and serum AMH levels. We noticed that women above the age of 35 years had lower serum levels of AMH than younger women before and after RAI treatment. Accordingly, after $^{131}$I administration, we observed reduced serum concentrations of AMH in both groups (younger and older). Therefore, RAI therapy used in women impacts the functional ovarian reserve that is independent of age. Therefore, we propose that women who plan to conceive, irrespective of their age, should have less aggressive PTC treatment, especially with low-risk PTC. Interestingly, the serum levels of other ovarian reserve markers (inhibin B and FSH) and AFC did not differ significantly before

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and after RAI treatment when we compared women below and over the age of 35 years. However, we should consider, particularly when interpreting negative results, that we examined a very small number of patients in the present study. Contrary to our findings, Evranos *et al.* did not observe any significant correlation between age and serum levels of AMH. However, they examined women younger than those who participated in our study (3).

Based on our results, we concluded that RAI treatment could potentially be responsible for the reduction of serum AMH levels. In the adjuvant/ablative treatment of thyroid cancer, the $^{131}$I isotope is used, which emits both beta and gamma radiation. This leads to radiation, that is, on the ovaries. It has been demonstrated that the dose of radiation received by the ovaries during treatment with $^{131}$I is 140 mGy with a given activity of 100 mCi (29). The high energy of beta radiation has the ability not only to damage one of the DNA strands but also to lead to the radiolysis of water. As a result, a lot of free radicals are formed in the most hydrated tissues. The most sensitive cells are growing and maturing (e.g. antral follicles). Increased oxidative stress levels (30) may cause severe oocyte damages, which may lead to an impairment of their fertilization capacity, probably caused by increased oxidative stress levels through apoptosis and DNA damage upregulations. The decreased developmental potential of oocytes from poorly vascularized follicles has also been attributed to low intrafollicular oxygenation. Oxidative stress has been shown to affect the midluteal corpus luteum and steroidogenic capacity in *in vitro* and *in vivo* studies. The correct follicular fluid microenvironment and oxidative stress status are also suggested to be responsible for a proper window to oocyte quality, fertility, and intracytoplasmic sperm injection. Furthermore, studies demonstrate that intensified lipid peroxidation in the preovulatory Graafian follicle might help maintain low levels of hydroperoxides inside follicles, suggesting an important role of oxidative stress balance in ovarian function (31). RAI likely lowers the total number of antral follicles, which would explain why decreased serum levels of AMH could be observed. However, further research is needed to clarify the exact pathogenetic mechanisms of this approach. Animal studies have shown that metformin might protect against the adverse effects of reactive oxygen species; thus, it could be a potential modulator to mitigate the effects of radiation on the body. An additional benefit is the protective nature of this drug, even when administered 24 h after radiation exposure, making it a potentially useful drug for use in counteracting the adverse effects of radiation (32). However, more studies of the effect of metformin on ovarian function in women treated with $^{131}$I are needed. Additionally, the effect of different doses of $^{131}$I on the ovarian reserve should also be assessed.

Based on the results of our research, it is not possible to determine the influence of RAI on the quality of oocytes. Moreover, there are no available tools for the assessment of oocyte quality at the moment. Individual AMH serum concentrations accurately reflect the size of the pool of antral follicles, and high serum levels of AMH are associated with a high antral follicular count (33). Additionally, the assessment of serum AMH can be useful as a predictor of oocyte yield following controlled ovarian stimulation and oocyte retrieval (4, 5). Therefore, the clinical implication of AMH reduction after RAI treatment could be associated with the need for ovarian stimulation, limited ovarian response to gonadotropins, and ovarian stimulation with higher doses of gonadotropins. However, future prospective studies focusing on clinical pregnancy outcomes and live birth rates in women after RAI treatment are also needed.

In this study, for the first time, we observed a decrease in AFC in USG after RAI treatment compared to baseline values. In the literature, a linear decline in AFC with age (median AFC decreases by 0.4 for every year) has been demonstrated (34). A decrease in AFC by 0.79 per year (95% CI −0.93, −0.64) has been observed in other studies (35). In our study, the median decrease in AFC was 2 during a 1-year observation (before treatment with RAI, the median AFC was 7; however, 1 year after, the RAI treatment median AFC was 5). Therefore, the obtained results indicate a significant influence of RAI therapy on AFC. In the ovaries, ovarian follicles of 5- to 8-mm diameter produce the majority of circulating AMH (19), and serum levels of AMH strongly correlate with AFC. In our study, we did not observe a significant relationship between serum levels of AMH and AFC. We can explain this by the small number of patients we recruited.

In this study, for the first time, we have shown that serum levels of another marker of the ovarian reserve (inhibin B) decreased during RAI treatment in premenopausal women with PTC relative to the baseline value; therefore, it could be a marker for estimating a decrease in the ovarian reserve in premenopausal women with PTC treated with RAI. However, the reference range of serum inhibin B levels remains unclear, and clinical cut-off values are not available. Moreover, due to different populations and detection kits in different studies and small sample sizes, the absolute values of inhibin B concentrations were also different. However, in the previous study, serum levels of inhibin B are positively correlated with AFC (6). Thus, we

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can assume that the decrease in serum levels of inhibin B in our study is *per se* associated with RAI treatment.

In this study, we did not observe significant differences between LH, FSH, and estradiol serum concentrations after RAI treatment and those at baseline in women with PTC. Similarly, Evranos *et al.* observed that serum concentrations of LH, FSH, and estradiol did not differ significantly before and after RAI treatment (3). Therefore, the above hormones could not be markers of the ovarian reserve in women with PTC treated with RAI.

The main limitation of this study is its relatively small sample size. However, this is the first prospective study assessing serum AMH levels and AFC and serum concentrations of FSH and inhibin B. Another limitation of this study is that follow-up was carried out for only 12 months. However, as demonstrated by van Velsen *et al.*, serum levels of AMH significantly dropped during the first year after RAI therapy and after that remained stable (17). The third limitation might be the absence of a control group; therefore, one might argue that the discovered decline of markers of the functional ovarian reserve over time reflects the biological decrease. However, in the 'Discussion' section, we have clearly shown that RAI treatment is associated with a more profound decrease in markers of the functional ovarian reserve. Accordingly, in previous studies, RAI's effect on the serum concentrations of AMH has been retrospectively assessed (13, 14), also without a control group (15), with a small number of participants (3), or included as a control group for women who had undergone thyroidectomy (13).

To conclude, we observed a decrease in serum concentrations of AMH, inhibin B, and AFC in USG 1 year after RAI treatment in premenopausal women with PTC relative to the baseline values. Therefore, these markers could be considered for the assessment of ovarian reserve in women with thyroid cancer. In the current recommendations for the management of women with DTC, nothing is said about women planning to conceive. The assessment of functional ovarian reserve markers prior to treatment with RAI and reduced doses of RAI should be considered in premenopausal women. Therefore, recommendations should include the determination of functional ovarian reserve markers because RAI treatment could be associated with a reduction of the total number of antral follicles.

**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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