ORIGINAL RESEARCH ARTICLE

Expression of thyroid antigens in the female reproductive system

DOI: 10.29063/ajrh2024/v28i9.13

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Abstract

Thyroid autoimmunity (TAI) has been linked to fertility disorders and pregnancy complications, even in euthyroid women. However, the exact pathophysiological mechanism underlying this association is not fully understood. This study seeks to investigate the expression of thyroid antigens within the human female reproductive system, potentially identifying targets for thyroid antibodies. Human biopsies of endometrium and follicular granulosa cells were collected and thyroperoxidase (TPO) and thyroglobulin (TG) expression was evaluated in these tissues by immunohistochemistry. Results showed, for the first time, the expression of TG protein and confirmed the presence of thyroid TPO in human endometrium and granulosa cells. Results suggest that TPO antibodies (TPOAbs) and TG antibodies (TGAbs) could interact with TPO and TG expressed in the reproductive system in patients with positive thyroid antibodies, thereby disrupting the function of TPO and TG and generating an inflammatory response, leading to fertility disorders and pregnancy complications. *(Afr J Reprod Health 2024; 28 [9]: 145-152).*

Keywords: Endometrium; granulosa cells; TPO; TG; thyroid autoimmunity; infertility

Résumé

L'auto-immunité thyroïdienne (AIT) est associée à des troubles de la fertilité et à des complications de grossesse, même chez les femmes euthyroïdiennes. Cependant, le mécanisme physiopathologique sous-jacent à cette association n'est pas entièrement élucidé. Cette étude vise à examiner l'expression des antigènes thyroïdiens dans le système reproducteur féminin humain, afin d'identifier des cibles potentielles pour les anticorps antithyroïdiens. Des biopsies d'endomètre et de cellules de granulosa ont été analysées pour l'expression de la thyroperoxydase (TPO) et de la thyroglobuline (TG) par immunohistochimie. Les résultats montrent, pour la première fois, l'expression de la TG et confirment la présence de la TPO dans l'endomètre et les cellules de granulosa humaines. Ces résultats suggèrent que les anticorps anti-TPO et anti-TG pourraient interagir avec la TPO et TG exprimés au niveau du système reproducteur des patientes présentant des anticorps thyroïdiens positifs, perturbant ainsi leur fonction et entraînant une réponse inflammatoire pouvant conduire à des troubles de la fertilité et des complications de grossesse. *(Afr J Reprod Health 2024; 28 [9]: 145-152).*

Mots-clés: : Endomètre; cellules de granulosa; TPO; TG; auto-immunité thyroïdienne; infertilité

Introduction

Thyroid hormones (TH) are essential for female reproductive health, significantly influencing ovarian function, folliculogenesis, and the development of uterine tissues. Through their receptors present on ovarian and oocytes surface, they support follicle growth and ovulation and collaborate with follicle-stimulating hormone (FSH) to regulate granulosa cells (GCs) proliferation and estrogen production[1]. TH are also crucial for endometrial receptivity, as they regulate the proliferation and vascularization of the endometrium and facilitate blastocyst adhesion[2].

Overall, TH are essential for fetal neurodevelopment and maternal metabolism throughout pregnancy[3]. Given the significant interplay between thyroid function and reproductive physiology, thyroid disorders are frequently associated with fertility disorders. Thyroid disorders are often autoimmune in nature, with thyroid autoimmunity (TAI) being the most common autoimmune disease, particularly among women of reproductive age[4,5]. TAI reflects a

breakdown in immunological tolerance, characterised by lymphocytic infiltration of the thyroid gland, resulting in the production of thyroid auto-antibodies and causing tissue damage that may disrupt thyroid function[6]. TAI affects 5-20% of women and recent data indicate a notable increase in cases over the past decade[7,8]. Higher prevalence rates are observed in low- to middle-income countries, especially in Africa[8]. The presence of antithyroid antibodies (ATA) in patient serum is the biological manifestation of TAI. The most frequent ATA are thyroid peroxidase antibodies (TPOAbs) and thyroglobulin antibodies (TGAbs). Specifically, TPOAbs are present in the serum of 90 to 95% of patients with TAI, while TgAbs are present in 70 to 80%[9]. ATA can also be found in the serum of 3-20% biochemically euthyroid patients[10]. Studies from the last three decades show that high levels of TPOAbs and TGAbs are associated with female fertility disorders and pregnancy complications, even in euthyroid patients and *independent of Thyroid-Stimulating Hormone (TSH) status*[11-13]. Specifically, the frequency of TAI appears to be higher in infertile women, women affected with endometriosis, Polycystic Ovarian Syndrome (PCOS), Diminished Ovarian Reserve (DOR) and in idiopathic infertility[14-20]. Furthermore, the presence of serum thyroid antibodies has been frequently associated with recurrent miscarriage (RM) and preterm birth[12,13,21]. Similarly, spontaneous abortion has been positively correlated with increased ATA, and a significantly lower pregnancy rate was found in clinically and biochemically euthyroid women with positive ATA[22,23]. Despite numerous studies establishing the link between fertility issues and ATA, the precise pathophysiological mechanism linking ATA with these conditions remains elusive. The mechanism by which antibodies directed against the thyroid gland might interfere with the initiation and development of pregnancy is not yet fully understood[24-26].

This study seeks to examine TPO and TG expression in the female reproductive system, with the aim of exploring the hypothesis that ATA may directly affect reproductive function through antigen-antibody interactions.

Methods

Study population and samples

The study was conducted at the Centre of Medically Assisted Reproduction (CMAR), Tiziri in Algiers, Algeria. The study enrolled women of childbearing age with regular menstrual cycles who sought assistance at the CMAR for male infertility undergoing In vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI). Only cases where infertility was attributed to male factors were included, and there were no instances of cancer included in the study. Data collection was performed by examining the patients' medical records. Using a Cornier pipelle, ten endometrial samples were collected by a gynecologist during the proliferative phase for the patients' routine gynecological check-up. After a pathologist confirmed the endometrial tissue was healthy, the samples were used for the study.

Controlled ovarian stimulation was conducted using an antagonist protocol. Recombinant FSH was administered starting on cycle day 2 and continued until ovulation induction. A GnRH antagonist (0.25 mg) was introduced on day 6 of stimulation and maintained until ovulation. Ovulation was triggered with either human chorionic gonadotropin or a 0.2 mg bolus of GnRH agonist. Oocyte retrieval was performed 36 hours post-trigger. Three mature follicles, each from a different patient, were aspirated. The cumulus cells surrounding the oocytes were then isolated and collected. Endometrial samples and cumulus cells were collected in formalin, paraffin-embedded, and sections were stained using hematoxylin and eosin for cytological examination. The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Issad Hassani University Hospital Center (Beni Messous, Algiers) and registered under

N°03/CE/2023. Written informed consent was obtained from patients for the use of their tissues in this study.

Immunohistochemistry

For immunochemistry analysis, five µm-thick paraffin sections were deparaffinized through series of xylene and ethanol washes. Antigen retrieval was performed by immersing the slides in pre-warmed citrate buffer and heated for 50 minutes in the microwave. Endogenous peroxidase activity was blocked using Peroxidase Suppressor (Pierce™ Peroxidase Detection Kit, Thermo Fisher Scientific, cat no: 36000) for 30 min in a humid chamber. Immunochemistry staining was performed using Thermofisher Thyroid Peroxidase Polyclonal Antibody (Thermo Fisher Scientific, cat no. PA581070) and Thyroglobulin Polyclonal Antibody (Thermo Fisher Scientific, cat no. PA5-82034). Primary antibodies were diluted at 1:100 for TPOAbs and 1:50 for TG using Thermofisher Universal Blocker Blocking Buffer (Pierce™ Peroxidase Detection Kit, Thermo Fisher Scientific, cat no: 36000) and incubated overnight at 4°C. Negative controls were obtained by omitting the primary antibodies. After washing, the slides were incubated with secondary antibody (Goat anti-Rabbit IgG Secondary Antibody, HRP Thermo Fisher Scientific, cat no. 31460) for 30 minutes. Staining was revealed using Metal Enhanced DAB (Metal Enhanced DAB Substrate KitThermo Fisher Scientific, cat no. 34065) for 10 minutes, and counterstaining was performed with hematoxylin. Images were taken using Olympus cellSens software.

Results

Characteristics of patients

Patient general data and biological results are presented in Table 1. Data are presented as mean±sd.

Immunohistochemistry

In the endometrium, TPO and TG expression was localized in the cytoplasm of luminal and glandular epithelial cells (Figure 1). TPO and TG expression was localized in the cytoplasm of the GCs (Figures 2 and 3).

Table 1: Patients' characteristics and laboratory assays. Data are presented as mean±sd

Parameters	N = 13
Mean age (years)	33.67 ± 2.31
Basal FSH (UI/l)	6.27 ± 1.50
Basal LH (UI/l)	6.12 ± 3.24
AMH (ng/ml)	2.08 ± 0.45
TSH (mIU/l)	1.46 ± 0.51
BMI (kg/m²)	24.59 ± 1.38

FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; AMH: Anti-Mullerian hormone; TSH: Thyroid-stimulating hormone; BMI: Body Mass Index.

Immunohistochemistry staining revealed the presence of TPO and TG in all of the examined samples of endometrium (Figure 1) and granulosa cells (Figures 2 and 3).

Discussion

The physiopathological mechanism linking elevated levels of ATA to fertility issues is not fully elucidated. Two competing theories suggest that (i) ATA act as biomarkers for an altered reserve of thyroid hormones, despite normal thyroid function tests; and (ii) ATA are indicators of a hostile immune environment, which would not only affect the thyroid gland[16]. Several accumulated studies show that TAI is associated with an imbalance of abnormal immune markers that may induce disruption in the establishment and progress of pregnancy[27,28]. The hypothesis suggesting an immune dysfunction, with TAI serving as a biomarker for immune abnormalities responsible for pregnancy complications, appears most plausible[29]. However, ATA could also directly affect the female reproductive system. Indeed, two recent studies demonstrated that human ovarian granulosa cells (GC), endometrium and placenta express TPO[30,31]. TPOAbs could therefore interact with TPO expressed in the reproductive system, disrupt the enzyme function, and trigger inflammatory response, thereby leading to potential fertility potential disorders.

This study identifies, for the first time, the expression of thyroglobulin (TG) protein and confirmed the presence of thyroid peroxidase (TPO) in human endometrium and GCs. Consequently, both anti-TPO and anti-TG antibodies could potentially interact with thyroid antigens within the

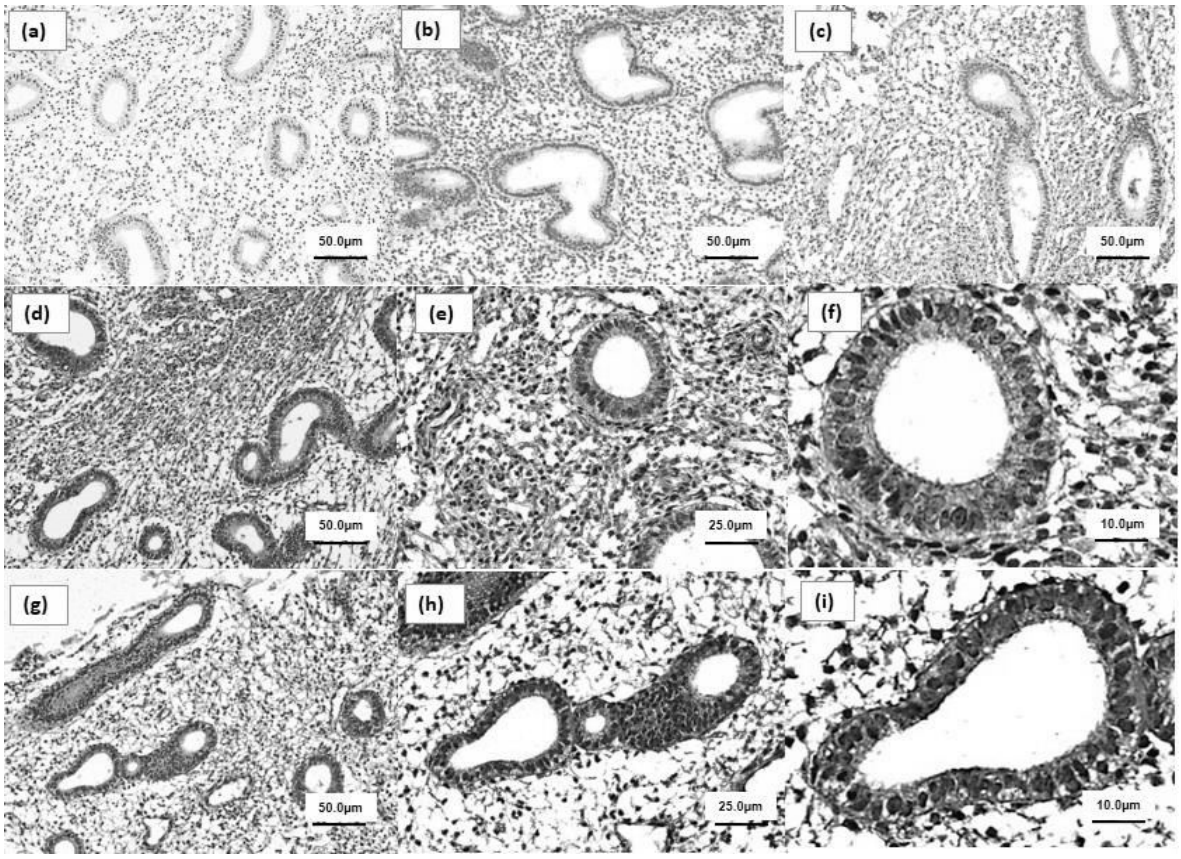

Figure1: Immunological staining of TPO and TG in human endometrium using anti-TPO and anti-TG. (a): H&E Staining of Endometrium.(b,c): negative controls of endometrium. (d,e,f): expression of TPO in endometrium. (g,h,i): expression of TG in endometrium

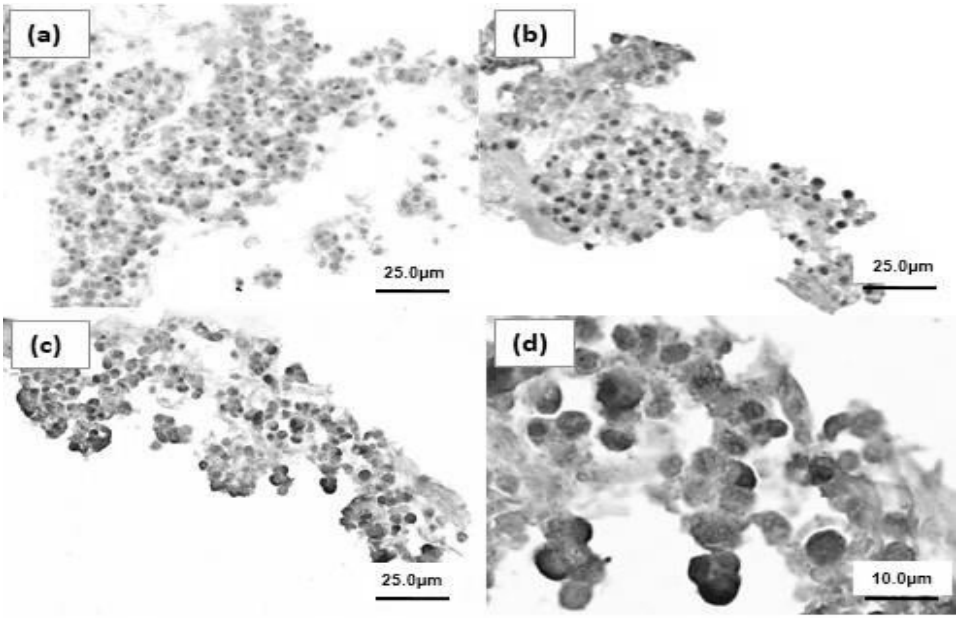

Figure 2: Immunological staining of TPO in human Granulosa Cells (GCs) using anti-TPO. (a): H&E Staining of GCs. (b): negative control of GCs. (c,d): expression of TPO in GCs

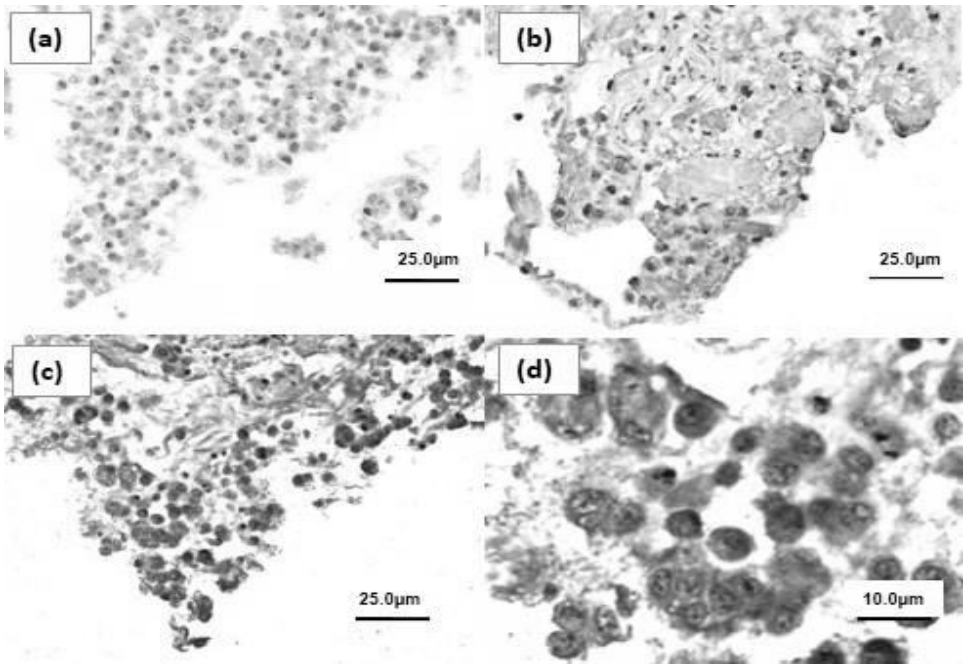

Figure 3: Immunological staining of TG in human Granulosa Cells (GCs) using anti-TG. (a): H&E Staining of GCs. (b): negative control of GCs. (c,d): expression of TG in GCs

reproductive system. In the endometrium, TPO and TG expression was localized in the cytoplasm of the surface epithelial cells and secretory glands (Figure 1). In an earlier study, TPOAbs were identified on the surface of pre-implantation embryos, and it has been demonstrated that both TPOAb and TgAb can freely cross the placental barrier[32,33]. An antigen-antibody interaction at the endometrium level is therefore possible. Similarly to their actions at the thyrocyte level, TPOAb might act through antibody-dependent cytotoxicity (ADCC) and C3 complement-mediated cytotoxicity, while TgAb could act via ADCC[6,34]. Consequently, it induces inflammation, impaired cellular function, and tissue damage. Here, it could potentially result in the disruption of embryonic implantation and placentation, and therefore lead to the infertility or obstetrical complications described[35].

In granulosa cells (GCs), TPO and TG were expressed in the cytoplasm (Figures 2 and 3). ATA, present in the follicular fluid, could act similarly to their actions at the thyrocyte level, as described above, and disturb the process of folliculogenesis, altering oocyte quality[36]. Furthermore, the expression of these antigens in the GCs supports the hypothesis proposed by Monteleone *et al.*, suggesting that the human ovarian follicle may be an independent thyroid-hormone producing unit[30]. This hypothesis is supported by the fact that key components necessary for thyroid hormone production, such as TPO, Tg, thyroid-stimulating hormone receptor (TSHR), Dual oxidases 1 and 2 (Duox-1, Duox-2) are expressed in granulosa cells[34,37]. This implies the possibility of local synthesis of thyroid hormones[38]. Since thyroid hormones play a direct role in folliculogenesis and ovarian function, TAI could lead to tissue-level thyroid hormone deficiency and therefore disrupt folliculogenesis and impact oocyte quality[39,40].

Finally, regarding woman with TAI resorting to ART, recent guidelines from the European Thyroid Association (ETA) recommend to screen systematically for TSH and ATA. ETA also suggests considering intra-cytoplasmic sperm injection (ICSI) for fertilization[41]. However, if ICSI may help to overcome the adverse effects of ATA on oocytes and embryos, it cannot prevent adverse effects of TAI on implantation and post-implantation embryos[42]. Further studies are needed to optimize the management of euthyroid patients with TAI undergoing ART.

Conclusion

This study isolated for the first time the expression of thyroglobulin protein and confirmed the presence of thyroid peroxidase (TPO) in human endometrium and granulosa cells. Consequently, in patients with positive TPOAbs and TGAbs, damage to reproductive organs expressing TPO and TG may occur. This damage can disrupt the processes of folliculogenesis, embryonic implantation, and placentation, ultimately contributing to fertility or obstetrical complications already associated with thyroid autoimmunity. These findings contribute to understanding the connection between anti-thyroid antibodies and fertility disorders. Further studies are needed to investigate the potential for local synthesis of thyroid hormones within the female reproductive system.

Funding

Co-funded by the European Union (ERC, RE3MODEL, 101042751). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them.

Conflicts of interest

None.

Authors contribution

Conceptualization: NB, NK, AO, SA. Project implementation: SA, NB, EMC, NGG Writing-original draft: SA Writing–review & editing: EMC, NGG, NB, NK. All authors mentioned in the article approved the manuscript.

Acknowledgements

We would like to express our gratitude to all the patients who participated in the study and to the staff of the Tiziri IVF Center for their assistance, without which this research would not have been possible. We are also deeply grateful to the anatomic pathologists Dr. H. Bouabbane and Prof. L. Kaci for their invaluable expertise and support in sample processing and analysis.

References

1. Mazzilli R, Medenica S, Di Tommaso AM, Fabozzi G, Zamponi V, Cimadomo D, Rienzi L, Ubaldi FM, Watanabe M, Faggiano A, La Vignera S and Defeudis G. The role of thyroid function in female and male infertility: a narrative review. J Endocrinol Invest. 2023;46(1):15–26. https://doi.org/10.1007/s40618-022-01883-7
2. Ren B and Zhu Y. A new perspective on thyroid hormones: crosstalk with reproductive hormones in females. Int J Mol Sci. 2022;23(5):2708. https://doi.org/10.3390/ijms23052708. PMID: 35269847; PMCID: PMC8911152.
3. Juneo FS, Ocarino NM and Serakides R. Thyroid hormones and female reproduction. Biol Reprod. 2018;99(5):907–21. https://doi.org/10.1093/biolre/ioy115.
4. Vissenberg R, Manders VD, Mastenbroek S, Fliers E, Afink GB, Ris-Stalpers C, Goddijn M and Bisschop PH. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Hum Reprod Update. 2015 May-Jun;21(3):378-87. doi: 10.1093/humupd/dmv004. PMID: 25634660.
5. McGrogan A, Seaman HE, Wright JW and De Vries CS. The incidence of autoimmune thyroid disease: a systematic review of the literature. Clin Endocrinol (Oxf). 2008;69(5):687-696. doi: 10.1111/j.1365-2265.2008.03338.x.
6. Bogusławska J, Godlewska M, Gajda E and Piekiełko-Witkowska A. Cellular and molecular basis of thyroid autoimmunity. Eur Thyroid J. 2022;11(1):e210024. doi: 10.1530/ETJ-21-0024.
7. Conrad N, Misra S, Verbakel JY, Verbeke G, Molenberghs G, Taylor PN, Mason J, Sattar N, McMurray J, McInnes IB, Khunti K and Cambridge G. Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort study of 22 million individuals in the UK. Lancet (London, England). 2023;401(10391):1878–1890. https://doi.org/10.1016/S0140-6736(23)00457-9
8. Hu X, Chen Y, Shen Y, Tian R, Sheng Y and Que H. Global prevalence and epidemiological trends of Hashimoto's thyroiditis in adults: A systematic review and meta-analysis. Front Public Health. 2022;10:1020709. doi: 10.3389/fpubh.2022.1020709. ISSN: 2296-2565.
9. Carvalho GA, Perez CL and Ward LS. The clinical use of thyroid function tests. Arq Bras Endocrinol Metabol. 2013;57(3):193-204.
10. Wémeau J-L. Les Maladies de la thyroïde. 2nd ed. Paris: Elsevier Masson, 2022. 352 p. ISBN 9782294775833.
11. Twig G, Shina A, Amital H and Shoenfeld Y. Pathogenesis of infertility and recurrent pregnancy loss in thyroid autoimmunity. J Autoimmun. 2012;38(2-3):J275-81.
12. Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J and Coomarasamy A. Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. BMJ. 2011 May

9;342:d2616. doi: 10.1136/bmj.d2616. PMID: 21558126; PMCID: PMC3089879.
13. Van Den Boogaard E, Vissenberg R, L and JA, Van Wely M, Van Der Post JA, Goddijn M and Bisschop PH. Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. Hum Reprod Update. 2011;17:605–619.
14. Unuane D, Velkeniers B, Anckaert E, Schiettecatte J, Tournaye H, Haentjens P and Poppe K. Thyroglobulin autoantibodies: is there any added value in the detection of thyroid autoimmunity in women consulting for fertility treatment? Thyroid. 2013 Aug;23(8):1022-8. doi: 10.1089/thy.2012.0562. PMID: 23405888; PMCID: PMC3752510.
15. Bucci I, Giuliani C, Di Dalmazi G, Formoso G and Napolitano G. Thyroid autoimmunity in female infertility and assisted reproductive technology outcome. Front Endocrinol. 2022;13:768363. https://doi.org/10.3389/fendo.2022.768363.
16. Tańska K, Gietka-Czernel M, Glinicki P and Kozakowski J. Thyroid autoimmunity and its negative impact on female fertility and maternal pregnancy outcomes. Front Endocrinol. 2023;13:1049665. https://doi.org/10.3389/fendo.2022.1049665
17. Wang X, Ding X, Xiao X, Xiong F and Fang R. An exploration on the influence of positive simple thyroid peroxidase antibody on female infertility. Exp Ther Med. 2018;16(4):3077-3081.
18. Bahreiny SS, Ahangarpour A, Amraei M, Mansouri Z, Pirsadeghi A, Kazemzadeh R, Javidan M, Karamali N, Bastani M-N and Dabbagh MR. Autoimmune thyroid disorders and polycystic ovary syndrome: Tracing links through systematic review and meta-analysis. J Reprod Immunol. 2024;163:104215. ISSN 0165-0378. https://doi.org/10.1016/j.jri.2024.104215.
19. Korevaar TIM, Mínguez-Alarcón L, Messerlian C, de Poortere RA, Williams PL, Broeren MA, Hauser R and Souter IC. Association of Thyroid Function and Autoimmunity with Ovarian Reserve in Women Seeking Infertility Care. Thyroid. 2018 Oct;28(10):1349-1358. doi: 10.1089/thy.2017.0582. Epub 2018 Aug 14. PMID: 29943679; PMCID: PMC6157366.
20. Chen CW, Huang YL, Tzeng CR, Huang RL and Chen CH. Idiopathic low ovarian reserve is associated with more frequent positive thyroid peroxidase antibodies. Thyroid. 2017;27(9):1194–200.
21. Busnelli A, Beltratti C, Cirillo F, Bulfoni A, Lania A and Levi-Setti PE. Impact of Thyroid Autoimmunity on Assisted Reproductive Technology Outcomes and Ovarian Reserve Markers: An Updated Systematic Review and Meta-Analysis. Thyroid. 2022 Sep;32(9):1010-1028. doi: 10.1089/thy.2021.0656. Epub 2022 Aug 29. PMID: 35819278.
22. Zhang J, Song Z, Yuan H and Cai ZH. The effects of metabolic indicators and immune biomarkers on pregnancy outcomes in women with recurrent spontaneous abortion: a retrospective study. Front Endocrinol (Lausanne). 2024;14:1297902. Published 2024 Jan 17. doi:10.3389/fendo.2023.1297902
23. Liu M, Wang D, Zhu L, Yin J, Ji X, Zhong Y, Gao Y, Zhang J, Liu Y, Zhang R and Chen H. Association of thyroid peroxidase antibodies with the rate of first-trimester miscarriage in euthyroid women with unexplained recurrent spontaneous abortion. Front Endocrinol. 2022;13:966565. https://doi.org/10.3389/fendo.2022.966565
24. Venables A, Wong W, Way M and Homer HA. Thyroid autoimmunity and IVF/ICSI outcomes in euthyroid women: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2020;18(1):120.
25. Leiva P, Schwarze JE, Vasquez P, Ortega C, Villa S, Crosby J, Balmaceda J and Pommer R. There is no association between the presence of anti-thyroid antibodies and increased reproductive loss in pregnant women after ART: a systematic review and meta-analysis. JBRA Assist Reprod. 2017;21(4):361–365.
26. Rao M, Zeng Z, Zhang Q, Su C, Yang Z, Zhao S and Tang L. Thyroid autoimmunity is not associated with embryo quality or pregnancy outcomes in euthyroid women undergoing assisted reproductive technology in China. Thyroid. 2023;33(3):380-388. doi: 10.1089/thy.2022.0184.
27. Kim NY, Cho HJ, Kim HY, Yang KM, Ahn HK, Thornton S, Park JC, Beaman K, Gilman-Sachs A and Kwak Kim J. Thyroid autoimmunity and the outcomes of assisted reproductive technology: a meta-analysis. Hum Reprod Update. 2022;28(3):277-292.
28. Lu H, Huang Y, Xin H, Hao C, Cui Y. The expression of cytokines IFN-γ, IL-4, IL-17A, and TGF-β1 in peripheral blood and follicular fluid of patients testing positive for anti-thyroid autoantibodies and its influence on in vitro fertilization and embryo transfer pregnancy outcomes. Gynecol Endocrinol. 2018;34(11):933–939.
29. Zhu Q, Xu QH, Xie T, Wang LL, Liu H, Muyayalo KP, Huang XB, Zhao SJ, Liao AH. Recent insights into the impact of immune dysfunction on reproduction in autoimmune thyroiditis. Clin Immunol. 2021;224:108663.Monteleone P, Faviana P and Artini PG. Thyroid peroxidase identified in human granulosa cells: another piece to the thyroid-ovary puzzle? Gynecol Endocrinol. 2017;33(7):574-576.
30. Rahnama R, Mahmoudi AR, Kazemnejad S, Salehi M, Ghahiri A, Soltanghoraee H, Vafaei S, Rezaei A and Zarnani AH. Thyroid peroxidase in human endometrium and placenta: a potential target for anti-TPO antibodies. Clin Exp Med. 2021;21(1):79-88.
31. Lee YL, Ng HP, Lau KS, Liu WM, O WS, Yeung WS and Kung AW. Increased fetal abortion rate in autoimmune thyroid disease is related to circulating TPO autoantibodies in an autoimmune thyroiditis animal model. Fertil Steril. 2009 May;91(5 Suppl):2104-9. doi: 10.1016/j.fertnstert.2008.07.1704. Epub 2008 Sep 6. PMID: 18774556.
32. Seror J, Amand G, Guibourdenche J, Ceccaldi PF and Luton D. Anti-TPO Antibodies Diffusion through the Placental Barrier during Pregnancy. PLoS ONE. 2014;9(1):e84647. https://doi.org/10.1371/journal.pone.0084647.

33. Aghajanova L, Lindeberg M, Carlsson IB, Stavreus-Evers A, Zhang P, Scott JE, Hovatta O and Skjöldebrand-Sparre L. Receptors for thyroid-stimulating hormone and thyroid hormones in human ovarian tissue. Reprod Biomed Online. 2009 Mar;18(3):337-47. doi: 10.1016/s1472-6483(10)60091-0. PMID: 19298732.
34. Matalon ST, Blank M, Levy Y, Carp HJ, Arad A, Burek L, Grunebaum E, Sherer Y, Ornoy A, Refetoff S, Weiss RE, Rose NR and Shoenfeld Y. The pathogenic role of antithyroglobulin antibody on pregnancy: evidence from an active immunization model in mice. Hum Reprod. 2003;18(5):1094–9.
35. Monteleone P, Parrini D, Faviana P, Carletti E, Casarosa E, Uccelli A, Cela V, Genazzani AR and Artini PG. Female infertility related to thyroid autoimmunity: the ovarian follicle hypothesis. Am J Reprod Immunol. 2011 Aug;66(2):108-14. doi: 10.1111/j.1600-0897.2010.00961.x. Epub 2011 Jan 18. PMID: 21241400.
36. Buck T, Hack CT, Berg D, Berg U, Kunz L and Mayerhofer A. The NADPH oxidase 4 is a major source of hydrogen peroxide in human granulosa-lutein and granulosa tumor cells. Sci Rep. 2019;9(1):3585.
37. Godlewska M and Banga PJ. Thyroid peroxidase as a dual active site enzyme: focus on biosynthesis, hormonogenesis and thyroid disorders of autoimmunity and cancer. Biochimie. 2019;160:34–45.
38. Brown EDL, Obeng-Gyasi B, Hall JE and Shekhar S. The thyroid hormone axis and female reproduction. Int J Mol Sci. 2023;24(12):9815.
39. Colella M, Cuomo D, Giacco A, Mallardo M, De Felice M and Ambrosino C. Thyroid hormones and functional ovarian reserve: systemic vs. peripheral dysfunctions. J Clin Med. 2020;9(6):1679.
40. Poppe K, Bisschop P, Fugazzola L, Minziori G, Unuane D and Weghofer A. 2021 European Thyroid Association Guideline on Thyroid Disorders prior to and during Assisted Reproduction. Eur Thyroid J. 2021 Feb;9(6):281-295. doi: 10.1159/000512790. Epub 2021 Jan 21. Erratum in: Eur Thyroid J. 2021 Jun;10(3):268. PMID: 33718252; PMCID: PMC7923920.
41. Medenica S, Garalejic E, Arsic B, Medjo B, Bojovic Jovic D, Abazovic D, Vukovic R and Zarkovic M. Follicular fluid thyroid autoantibodies, thyrotropin, free thyroxine levels and assisted reproductive technology outcome. PLoS One. 2018 Oct 29;13(10):e0206652. doi: 10.1371/journal.pone.0206652. PMID: 30372494; PMCID: PMC6205652.