Sanja Medenica[1], Miloš Žarković[2]

TIROIDNA AUTOIMUNOST I REPRODUKCIJA – BIDIREKCIONA VEZA KOJA NASTAVLJA DA INTRIGIRA

Sažetak: Infertilitet predstavlja ozbiljan ne samo zdravstveni već i psiho-socijalni problem današnjice čija učestalost u svetu raste. Tiroidna autoimunost (TAI) je najčešće oboljenje tiroidne žlezde u reproduktivnom periodu koje može uticati i na sponatno začeće, kao i na začeće putem metoda asistirane reprodukcije (ART), ali i na održavanje trudnoće, i biti uzrok brojnih maternalnih i fetalnih komplikacija. Brojne publikacije postoje na temu mehanizama povezanosti TAI i reprodukcije, uz pitanje da li su tiroidna autoantitela isključivo tkivno specifična antitela, da li i kada primeniti suplementaciju levotiroksinom, te da su nam potrebna fundamentalnija istraživanja o direktnom učinku tiroidnih autoantitela, počev od folikulogeneze do embriogeneze i implantacije, kao i postimplantacionog razvoja embriona, ali i sastava folikularne tečnosti kao mikrosredine od enormog značaja za maturaciju jajne ćelije u kojoj tiroidna autoantitela dospevaju preko krvno-folikularne barijere.

Ključne reči: tiroidna autoimunost, infertilitet, asistirana reprodukcija, folikularna tečnost

Uvod

Infertilitet predstavlja ozbiljan ne samo zdravstveni već i psiho-socijalni problem današnjice čija učestalost u svetu raste, obuhvatajući 8–12% parova širom sveta (1). S obzirom na to da su poremećaji tiroidne funkcije veoma česti u reproduktivnom periodu žene za očekivati je da se ova dva entiteta prepliću. Tiroidna autoimunost (TAI) je najčešće oboljenje tiroidne žlezde u reproduktivnom periodu sa učestalošću od 5 do 20% (2). Sa potrebom prevazilaženja problema infertiliteta raste potreba za

[1] Odeljenje za endokrinologiju, Interna klinika, Klinički centar Crne Gore, Medicinski fakultet, Univerzitet Crne Gore, Podgorica, Crna Gora

[2] Odeljenje za oboljenja štitaste žlezde, Klinika za endokrinologiju, dijabetes i bolesti metabolizma, Univerzitetski klinički centar Srbije, Medicinski fakultet, Univerzitet u Beogradu, Beograd, Srbija

korišćenjem metoda asistirane reprodukcije (ART), a uticaj TAI na spontano začeće, kao i ishode ART-a ostavlja brojne dileme nerešene.

Tiroidna autoimunost i trudnoća – imunološka osnova, potencijalni mehanizmi povezanosti, uloga tiroidnih hormona i moguće komplikacije u vezi sa spontanim začećem

TAI podrazumeva poremećaj imunološke tolerancije na sopstvene antigene. Glavni antigeni u razvoju TAI su tireoglobulin (Tg), tiroidna peroksidaza (TPO) i receptor za tireotropin (TSHR), ređe zastupljeni antigeni natrijum/jodid simporter (NIS) i druge komponente tireocita (2). Antitela na TPO (antiTPO At) i antitela na Tg (antiTg At) ostvaruju citotoksičnu aktivnost (3), tako da razmatranje TAI u kontekstu efekta iste na koncepciju, dominantno se odnosi na prisustvo antiTPO At i/ili antiTg At (1). Pored humoralnog, i celularni imunitet ima veliki značaj u nastanku TAI, a citokini su osnov autoimunog odgovora i imaju niz direktnih i indirektnih efekata koji u konačnici mogu dovesti do destruktivnog tiroiditisa i deterioracije tiroidne funkcije (4). Nemogućnost koncepcije kod žena sa TAI povezana je sa oštećenim humoralnim i celularnim imunim odgovorom (5, 6). Regulatorne T ćelije (Tregs) su podskup CD (*cluster of differentiation*) 4 + T ćelija, čija je uloga u održavanju tolerancije suzbijanjem imunološkog odgovora (7). Ipak, navedene ćelije su disfunkcionalne u TAI (7). U ranim fazama gestacije dolazi do značajnog povećanja *natural killer* (NK) ćelija u decidui usled preraspodele iz periferne krvi, a pod uticajem seksualnih steroida (8). Zapaža se povezanost promena broja i funkcije NK ćelija u neuspeloj koncepciji, ali i rekurentnim spontanim pobačajima, što upućuje na značaj istih u imunomodulaciji između majke i fetusa (9).

Brojne su publikacije na temu povezanosti TAI i subfertiliteta žene, neželjenih ishoda trudnoće začete spontano, kao i udruženost sa brojnim maternalnim i fetalnim komplikacijama (1, 10). Nekoliko hipoteza je predloženo da pojasni bidirekcionu vezu TAI i infertiliteta (11). Prva hipoteza govori u prilog podatka da je gubitak trudnoće posledica autoimune neravnoteže koji rezultuje odbacivanjem 'fetalnog grafta', a tiroidna autoantitela posledica generalizovanog autoimunog odgovora. TAI je često udružena sa tiroidnom disfunkcijom, te se druga hipoteza odnosi na blagu deficijenciju tiroidnih hormona ili smanjenu sposobnost tiroidne žlezde da se adaptira na hormonske promene u trudnoći u miljeu TAI. TAI je povezana sa smanjenom ovarijalnom rezervom i sniženim vrednostima anti-Mullerian hormona (AMH) (12), te sama može biti uzrok odložene koncepcije. Uzimajući u obzir značaj folikularne tečnosti, kao mikrosredine za maturaciju jajnih ćelija, hipoteza folikula jajnika ističe značaj prisustva tiroidnih autoantitela u folikularnoj tečnosti, koja se ne generišu u istoj, nego prelaze krvno-folikularnu barijeru i utiču direktno na kvalitet jajne ćelije

(13). Pokazano je prisustvo TPO na zrelim granuloza ćelijama, te antiTPO At deluje direktno na maturaciju jajne ćelije, što bi moglo objasniti uticaj lokalne autoimunosti na nivou folikula jajnika. Jedan od najnovijih predloženih modela koji opisuje razvojne stadijume TAI i direktan uticaj na jajnik objašnjava da se isti ostvaruje kroz dve faze: ranu, u kojoj je još uvek intaktna i dovoljna proizvodnja tiroidnih hormona na stimulaciju humanim horionskim gonadotropinom (hCG) i kasnu fazu u kojoj ipak dolazi do deterioracije tiroidne funkcije i neuspevanja da se adekvatno odgovori na stimulaciju hCG uzrokujući suboptimalnu proizvodnju tiroidnih hormona i delujući i na folikule jajnika, ali i ostala reproduktivna tkiva (14). Autoantitela na zonu pelucidu igraju važnu ulogu u reprodukciji žena sa TAI (15).

Tokom gestacije smanjuje se titra antitela do 60%, ali kod inicijalno TAI eutiroidnih žena postoji mogućnost progresije ka hipotiroidizmu tokom trudnoće i porasta TSH preko 4 mIU/L, a 33–50% žena koje imaju pozitivna tiroidna autoantitela u prvom trimestru će razviti postpartalno tiroiditis (16). Kada govorimo o TSH zavisnim mehanizmima povezanosti ova dva entiteta treba imati na umu da je TAI često udružena sa porastom serumske koncentracije TSH (17). Uticaj na maturacije jajne ćelije tiroidni hormoni ostvaruju preko svojih receptora koji su prisutni na ovarijalnom tkivu (16), međutim, kod spontanih i rekurentnih pobačaja zapažena je njihova nishodna regulacija (18). Njihov uticaj ne samo da se ogleda u folikulogenezi, nego i receptivnosti endometrijuma, čime se može menjati šansa za implantaciju (19). Iako je pokazano da TSH zavisnim mehanizmima žene sa hipotiroidizmom, pa i subkliničkom formom, imaju manju šansu da ostvare trudnoću, prirodnim putem ali i putem metoda ART-a, poslednje velike studije nisu potvrdile značaj prekoncepcijske primene levotiroksina na ishode trudnoće kod eutiroidnih žena sa TAI i problemom infertiliteta ili rekurentnih pobačaja (1). Shodno tome, Evropska tiroidna asocijacija (ETA) je dala kao preporuku da žene sa problemom subfertiliteta i prisutnim antiTPO At treba lečiti levotiroksinom ukoliko je TSH viši od 4,0 mIU/L u cilju postizanja vrednosti TSH manje od 2,5 mIU/L, dok bi žene sa TAI i TSH više od 2,5 mIU/L mogle biti lečene u cilju optimizacije razvoja embriona (1). Ipak, još uvek je nedovoljno dokaza da pri vrednostima TSH višim od 2,5 mIU/L, uz normalan fT4, postoji veći rizik od infertiliteta, dok je rizik od infertiliteta pri serumskim koncentracijama TSH višim od 4 mIU/L pokazan u brojnim studijama (20). I opet, iako negde u međuvremenu zapostavljena, antiTg At dobijaju svoje, prema rezultatima studija, zasluženo mesto. Ukoliko su antiTPO At negativna u slučaju vrednosti TSH više od 2,5 mIU/L, merenje antiTgAt ili pak sonografske karakteristike štitaste žlezde, koje upućuju na hronični autoimuni proces, mogu biti dovoljne za odluku o terapiji (1). Prema najnovijim smernicama Američke tiroidne asocijacije (ATA), antiTPO At treba meriti kod svih trudnica sa TSH višim od 2,5 mU/L. Kod svih žena sa TSH višim od 10,0 mU/L treba započeti lečenje čak i kada su slobodne frakcije tiroidnih hormona unutar referentnog opsega. Žene sa prisutnim antiTPO At treba lečiti ako je

TSH iznad referentnog opsega specifičnog za trudnoću i može se razmotrii lečenje ako je koncentracija TSH viša od 2,5 mU/L i ispod gornje granice trimestar specifičnih referentnih vrednosti. Žene bez antiTPO At mogu biti lečene ako imaju koncentraciju TSH iznad gornje referentne trimestar specifične vrednosti i ispod 10,0 mU/L, ali ne treba da se leči ukoliko je TSH u okviru referentnih trimestar specifičnih vrednosti ili ispod 4,0 mU/L ukoliko trimestar specifične vrednosti nisu dostupne (10).

Još od prve publikacije na temu povezanosti TAI i dvostrukog rizika od nastanka spontanog pobačaja Stagnaro Green-a i saradnika (21), literatura upućuje na vezu TAI i brojnih maternalnih komplikacija, među kojima se izdvajaju spontani pobačaj i prevremeni porođaj (22–30), ali se opisuje i značaj TAI u razvoju neonatalnih komplikacija poput mrtvorođenosti, male porođajne telesne mase, neonatalnog distresa i drugih (31). Majke sa antiTPO At su rađale decu veću za gestacijsku dob (32). Usporeniji motorni i intelektualni razvoj dece majki sa TAI (33), kao i gubitak senzorineuralnog sluha kod dece TAI pozitivnih žena je zapažen u literaturi (34). U fokusu istraživanja se stavljaju i antiTg At, u smislu potencijalnog uticaja istih na perceptualne performanse i motoričke rezultate (35).

Tiroidna autoimunost i trudnoća ostvarena asistiranom reprodukcijom

Tiroidna autoantitela su prepoznata kao nezavisni marker neuspelih ishoda vantelesne oplodnje (VTO) (36), koja utiču na folikulogenezu, fertilizaciju, embriogenezu, implantaciju (37). Rezultati studija upućuju da je TAI udružena sa neželjenim ishodima VTO (11,38–39), s posebnim osvrtom na spontani pobačaj i prevremeni porođaj, kao i nižu stopu živorođenosti (40). Ipak, nisu rezultati svih studija konkordantni (41, 42). Smanjena ovarijalna rezerva i TAI se često pominju u istom kontekstu, iako sam patofiziološki mehanizam povezanosti nije u potpunosti razjašnjen, ali je TSH nezavistan (43). Kako se pretpostavlja, tiroidna autoantitela prolaze krvno-folikularnu barijeru, ista mogu ispoljiti direktan negativan uticaj na rastući folikul i jajnu ćeliju (44), ali mogu imati uticaj i na postimplantacioni razvoj embriona (45). U novije vreme, antiTg At dobijaju opet na značaju, jer ne samo *in vitro* nego i *in vivo* istraživanja pokazuju da prisustvo ovih antitela može biti razlog porasta stope fetalne apsorpcije (46, 47), dakle, tiroidna autoantitela potencijalno su razlog odbacivanja embriona nakon embriotransfera, tj. implantacije delovanjem na feto-placentalnu jedinicu (48). Receptori za spermatozoide nalaze se na zoni pelucidi, koja okružuje jajnu ćeliju tokom ovulacije, te se pretpostavlja da antitela na zonu pelucidu koja se nalaze u folikularnoj tečnosti mogu biti uzrok infertiliteta, sprečavajući kontakt jajne ćelije i spermatozoida (13). Zato se i veruje da bi primena metode fertilizacije intracitoplazmatskom injekcijom spermatozoida (ICSI) mogla biti metod izbora, jer je idealan način da se prevaziđe postojeća barijera (1), čak i u situacijama

kada antitela utiču na kvalitet jajne ćelije (47). Postoje dokazi da bi antitela na zonu pelucidu antitela mogla nastati kao rezultat ponavljanih mikrotrauma usled repunkcija folikula u procedurama VTO (49). Ovarijalna stimulacija (OS), kao deo VTO procedure, uzrokuje porast serumskog estradiola do vrednosti 4.000–6.000 ng/L, sledstveno porast globulina koji vezuje tiroksin (TBG) i smanjenje slobodnih frakcija tiroidnih hormona, uz efekat na tireotropin-oslobađajući hormon (TRH), što kod oko 30% žena može da dovede do porasta nivoa TSH u serumu preko 2,5 mIU/L tokom VTO ciklusa, sa trajanjem 1 do 3 meseca (1, 50). Stopa pacijenata sa TSH višim od 2,5 mIU/L, kao i amplituda porasta TSH izraženiji su kod hipotiroidnih žena na supstituciji verovatno kao posledica smanjene mogućnosti adaptacije tiroidne žlezde na povećanu aktivnost tokom OS (51). Još jedan efekat OS na funkciju tiroidne žlezde koji treba uzeti u obzir je onaj koji se odnosi na završnu maturaciju jajne ćelije, kada se pik TSH očekuje nakon nedelju dana od primenjene injekcije humanog horionskog gonadotropina (hCG) (52). ETA preporučuje da se kod žena sa TAI i suplementacijom levotiroksinom ili započinjanjem istog, a koje su u postupku VTO nakon OS provera, TSH uradi, počevši od drugog merenja hCG ako je žena trudna, što je oko 6 nedelja nakon početka stimulacije ili 3 nedelje nakon indukcije ovulacije. Prilagođavanje doza levotiroksina se preporučuje kod žena koje su već bile na terapiji pre OS, u cilju održavanja vrednosti serumskog TSH nižim od 2,5 mIU/L. Predlaže se lečenje TAI pozitivnih žena sa nivoima TSH preko 2,5 i ispod 4,0 mIU/L ili gornje referentne granice malom dozom levotiroksina (obično 25–50 mcg dnevno) pre OS, posebno u situacijama rekurentnih pobačaja, kod žena preko 35 godina starosti, kao i ovarijalnih uzroka infertiliteta. Preporučuje se lečenje TAI pozitivnih žena sa TSH višim od 4,0 mIU/L ili iznad gornje referentne granice pre OS da bi se nivo TSH održavao nižim od 2,5 mIU/L, kao i lečenje TAI negativnih žena sa nivoom TSH preko 4,0 mIU/L ili preko gornje referentne granice pre OS (1). Primena dugog protokola stimulacije agonistima gonadotropin-oslobađajućeg hormona dovodi do porasta stope kliničke trudnoće, naime, primena ovog protokola pozitivno korelira sa serumskim estradiolom na dan završne injekcije i praćena je nižim serumskim vrednostima TSH pre započinjanja procedure (53).

Dok su pojedine studije pokazale da primena levotiroksina može da dovede do poboljšanja stope živorođenosti i smanjenja stope pobačaja kod žena sa TAI u postupku VTO (53, 54), rezultati novijih velikih studija ih ne podržavaju. POSTAL nije pokazala značaj primene levotiroksina u smislu smanjenja stopa spontanih pobačaja, kliničke trudnoće i živorođenosti (55). Skorašnja meta analiza je istakla da suplementacija levotiroksinom nema statistički značajan uticaj na stopu kliničke trudnoće, živorođenosti, prevremenog porođaja kod žena sa subkliničkom hipotireozom i/ili TAI u postupku VTO, ali se ipak zapaža smanjenje stope spontanih pobačaja (56). TABLET je još jedna velika studija koja nije uspela da pokaže efikasnost primene levotiroksina u TAI pozitivnih žena u cilju povećanja stope živorođenosti (57). Re-

zultati T4-LIFE studije pokazuju da, u poređenju sa placebom, lečenje levotiroksinom nije rezultiralo većom stopom živorođenosti kod eutireoidnih antiTPO At pozitivnih žena sa rekurentnim pobačajima, na osnovu čega autori ne savetuju rutinsku upotrebu levotiroksina kod antiTPO At pozitivnih žena rekurentnim pobačajima i normalnom tiroidnom funkcijom (58).

Zaključak

Deluje da iz dana u dan imamo sve više podataka na temu povezanosti TAI i reprodukcije i brojnih konsekvenci do kojih ova veza dovodi, preporuka o (ne)lečenju, korišćenju metoda ART-a, odabira protokola za OS, kao i adekvatnog metoda fertilizacije, uticaja tiroidnih autoantitela na maturaciju jajne ćelije, razvoj embriona, implantaciju istog, te na stopu uspešnosti primenjene metode sve u cilju povećanja stope živorođenosti. Međutim, treba imati na umu niz kofaktora, kao što su godine života, prekomerna uhranjenost ili gojaznost, životne navike i brojne druge, koje mogu udruženo ometati koncepciju ili tok trudnoće i, naravno, istovremeno, ukoliko je potrebno lečiti i partnera. Ipak, svedoci smo da, i pored svega što do sada znamo, bidirekciona veza TAI i reprodukcije nastavlja da intrigira uz pitanje da li su zaista tiroidna autoantitela odraz generalizovanog imunog odgovara, a ne isključivo tkivno specifična antitela, te da su nam potrebna fundamentalnija istraživanja o njihovom direktnom učinku počev od folikulogeneze do embriogeneze i implantacije, kao i postimplantacionog razvoja embriona, ali i sastava folikularne tečnosti kao mikrosredine od enormnog značaja za maturaciju jajne ćelije u kojoj tiroidna autoantitela dospevaju preko krvno-folikularne barijere.

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Sanja Medenica[1], Miloš Žarković[2]

THYROID AUTOIMMUNITY AND REPRODUCTION – BIDIRECTIONAL RELATIONSHIP THAT CONTINUES TO INTRIGUE

Abstract: Today, infertility is not only a serious health but also a psycho-social problem, one that is on the rise in the world. Thyroid autoimmunity (TAI) is the most common disease of the thyroid gland in the reproductive period, which can affect spontaneous conception as well as conception through assisted reproduction technology (ART), but also the maintenance of healthy pregnancy. It can also cause numerous maternal and fetal complications. There is a wide array of publications on the topic of the mechanisms of association between TAI and reproduction, with the question of whether thyroid autoantibodies are solely tissue-specific antibodies, whether and when to start levothyroxine treatment, and that we require more fundamental research on the direct effect of thyroid autoantibodies starting from folliculogenesis to embryogenesis and implantation as well as the post-implantation embryo development, but also the composition of the follicular fluid as a microenvironment of enormous importance for the maturation of the oocytes which thyroid autoantibodies reach via the blood-follicle barrier.

Keywords: thyroid autoimmunity, infertility, assisted reproduction, follicular fluid

Introduction

Today, infertility is not only a serious health but also a psycho-social problem, one that is on the rise in the world, affecting 8-12% of couples worldwide (1). Given that disorders of thyroid function are very common in a female reproductive period, it

[1] Department of Endocrinology, Internal Medicine Clinic, Clinical Center of Montenegro, School of Medicine, University of Montenegro, Podgorica, Montenegro

[2] Department of Thyroid Gland Diseases, Ckinic for Endocrinology, Diabetes, and Metabolic Disorders, University Clinical Center of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia

is to be expected that these two entities are intertwined. Thyroid autoimmunity (TAI) is the most common disease of the thyroid gland in the reproductive period with an incidence of 5% to 20% (2). Along with the need to overcome the issue of infertility, the need to use technologies of assisted reproduction (ART) grows as well, while the influence of TAI on spontaneous conception as well as the outcomes of ART leaves numerous dilemmas unresolved.

Thyroid autoimmunity and pregnancy-immunological basis, potential mechanisms of association, role of thyroid hormones, and possible complications related to spontaneous conception

TAI is an immune tolerance disorder to self-antigens. The main antigens in the development of TAI are thyroglobulin (Tg), thyroid peroxidase (TPO) and thyrotropin receptor (TSHR), less commonly represented antigens sodium/iodide symporter (NIS), and other thyrocyte components (2). TPO antibodies (TPOAbs) and Tg antibodies (TgAbs) achieve cytotoxic activity (3), therefore, placing TAI in the context of its effect on conception is predominantly related to the presence of TPOAbs and/or TgAbs (1). In addition to humoral, cellular immunity also plays a great role in the development of TAI. Cytokines are the basis of the autoimmune response and have a series of direct and indirect effects that can ultimately lead to destructive thyroiditis and deterioration of thyroid function (4). Infertility in women with TAI is associated with impaired humoral and cellular immune response (5,6). Regulatory T cells (Tregs) are a subset of CD(*cluster of differentiation*)4^+ T cells, whose role is to maintain tolerance by suppressing the immune response (7). However, these cells are dysfunctional in the case of TAI (7). In the early stages of gestation, there is a significant increase in natural killer (NK) cells in the decidua due to redistribution from the peripheral blood, under the influence of sex steroids (8). A link is noted between changes in the number and function of NK cells in failed conception, but also in recurrent spontaneous abortions, which points to their importance in immunomodulation between mother and fetus (9).

There is a wide array of publications on the topic of the link between TAI and female subfertility, adverse pregnancy outcomes in spontaneous conceptions, as well as the connection to numerous maternal and fetal complications (1,10). Several hypotheses have been proposed to explain the bidirectional relationship between TAI and infertility (11). The first hypothesis speaks in favor of the fact that pregnancy loss is a consequence of an autoimmune imbalance that results in the rejection of the 'fetal graft', and thyroid autoantibodies are a consequence of a generalized autoimmune response. TAI often occurs along with thyroid dysfunction, so another hypothesis refers to a mild deficiency of thyroid hormones or a reduced ability of the thyroid gland to adapt to hormonal changes during pregnancy in the

context of TAI. TAI is associated with a diminished ovarian reserve and low levels of the Anti-Mullerian hormone (AMH) (12), and may itself be the cause of delayed conception. Considering the importance of follicular fluid as a microenvironment for egg cell maturation, the ovarian follicle hypothesis highlights the importance of the presence of thyroid autoantibodies in follicular fluid, which are not generated in the follicular fluid but cross the blood-follicle barrier and directly affect the quality of the oocyte (13). The presence of TPO on mature granulosa cells was shown, and TPOAbs directly affects oocyte maturation, which could explain the influence of local autoimmunity at the level of ovarian follicles. One of the latest proposed models that describe the development stages of TAI and the direct impact on the ovary clarifies that it is achieved through two phases: an early phase in which thyroid hormone production is still intact and sufficient for stimulation by human chorionic gonadotropin (hCG) and a late phase marked by a decline in thyroid function and a failure to adequately respond to hCG stimulation, causing suboptimal production of thyroid hormones and affecting ovarian follicles and other reproductive tissues (14). Zona pellucid autoantibodies play an important role in the fertility of women with TAI (15).

During pregnancy, the antibody titer decreases by up to 60%, but in initially TAI euthyroid women there is a possibility of it turning into hypothyroidism during pregnancy and an increase in TSH over 4 mIU/L, while 33%-50% of women who have positive thyroid autoantibodies in the first trimester will develop postpartum thyroiditis (16). When we talk about TSH-dependent mechanisms of connection between these two entities, it should be kept in mind that TAI often appears along with an increase in the serum concentration of TSH (17). Thyroid hormones influence oocyte maturation through their receptors, which are present in the ovarian tissue (16), however, in spontaneous and recurrent miscarriages, their consequent regulation has been observed (18). Their influence is not only reflected in folliculogenesis but also in endometrial receptivity, which can change the chances of implantation (19). Although it has been shown that, through TSH-dependent mechanisms, women with hypothyroidism, including subclinical hypothyroidism, have a lower chance of getting pregnant, either naturally or through ART, recent meta-analysis have not confirmed the importance of preconception administration of levothyroxine on pregnancy outcomes in euthyroid women with TAI, and those who have had fertility issues, or recurrent miscarriages (1). Accordingly, the European Thyroid Association (ETA) recommended that women who suffer from subfertility and have TPOAbs should be treated with levothyroxine if TSH is higher than 4.0 mIU/L with the aim of attaining a TSH value lower than 2.5 mIU/L, while women with TAI and TSH higher than 2.5 mIU/L could be treated to optimize embryo development (1). However, there is still insufficient evidence that at TSH values higher than 2.5 mIU/L with normal fT4 there is a higher infertility risk, while numerous studies have shown that there is a risk of infertility at serum TSH concentrations higher

than 4 mIU/L (20). Once again, although neglected in the meantime, TgAbs get their deserved place, according to study results. If TPOAbs are negative in the case of a TSH value higher than 2.5 mIU/L, the measurement of TgAbs or sonographic characteristics of the thyroid gland that point to a chronic autoimmune process may be sufficient to determine the appropriate treatment (1). According to the latest guidelines of the American Thyroid Association (ATA), TPOAbs should be measured in all pregnant women with a TSH higher than 2.5 mU/L. In all women with a TSH higher than 10.0 mU/L, treatment should be started even when the free fractions of thyroid hormones are within the reference range. Women with TPOAbs should be treated if TSH is above the pregnancy-specific reference range and treatment may be considered if the TSH concentration is higher than 2.5 mU/L and below the upper limit of trimester-specific reference values. Women without TPOAbs can be treated if they have a TSH concentration higher than the upper trimester-specific reference value and lower than 10.0 mU/L, but they should not be treated if the TSH is within the trimester-specific reference values or lower than 4.0 mU/L - if trimester-specific values are not available (10). Ever since Stagnaro Green and associates wrote about the topic of the relationship between TAI and the double risk of spontaneous abortion(21), the literature has been pointing out the link between TAI and numerous maternal complications, including spontaneous abortion and premature birth (22-30), while also describing the importance of TAI in the development of neonatal complications such as stillbirth, low birth weight, neonatal distress and others (31). TPOAb-positive mothers gave birth to large for gestational age babies (32). Literature also notes that TAI-positive women may give birth to children with slower motor skills and intellectual development (33), as well as sensorineural hearing loss (34). TgAb is also the focus of research, in terms of their potential impact on perceptual performance and motor results (35).

Thyroid autoimmunity and pregnancy achieved by assisted reproductive technology

Thyroid autoantibodies have been recognized as an independent marker of unsuccessful IVF outcomes (36), affecting folliculogenesis, fertilization, embryogenesis, and implantation (37). Study results indicate that TAI is also linked with adverse outcomes of IVF (11,38-39), with special reference to miscarriage and premature birth, as well as a lower live birth rate (40). However, not all study results are concordant (41,42). Diminished ovarian reserve and TAI are often mentioned in the same context, although the pathophysiological mechanism of the link between them is not fully explained, but it is TSH-independent (43). As it is assumed that thyroid autoantibodies cross the blood-follicle barrier, they can have a direct negative effect on the growing follicle and egg cell (44), but also an effect on the post-implantation

development of the embryo (45). In recent times, TgAb is gaining importance again, because not only in vitro but also in vivo studies show that the presence of these antibodies can cause an increase in the rate of fetal absorption (46,47), so thyroid autoantibodies are potentially the reason for embryo rejection after embryo transfer i.e. implantation by stimulating the fetoplacental unit (48). Sperm receptors are located on the zona pellucida, which surrounds the oocyte during ovulation, and it is assumed that the zona pellucida antibodies found in the follicular fluid may be the cause of infertility, preventing the contact between the oocyte and spermatozoids (13). That is why it is believed that the application of the fertilization method by intracytoplasmic sperm injection (ICSI) could be used because it is the ideal way to overcome the existing barrier (1), even in situations where antibodies affect the quality of the oocyte (47). There is evidence that zona pellucida antibodies may arise as a result of repeated microtraumas due to follicular punctures in IVF procedures (49). Ovarian stimulation (OS), as part of the IVF procedure, causes an increase in serum estradiol to a value of 4,000–6,000 ng/L, resulting in an increase in thyroxine-binding globulin (TBG) and a decrease in free fractions of thyroid hormones, affecting thyrotropin-releasing hormone (TRH), which can lead to an increase in the level of TSH in the serum higher than 2.5 mIU/L during the IVF cycle, with a duration of 1 to 3 months, in about 30% of women (1.50). The number of patients with TSH higher than 2.5 mIU/L as well as the amplitude of TSH increase is higher in hypothyroid women on replacement therapy, possibly as a consequence of the reduced ability of the thyroid gland to adapt to increased activity during OS (51). Another effect of OS on thyroid function that should be taken into account is related to the final oocyte maturation, when the TSH peak is expected one week after the administered injection of human chorionic gonadotropin (hCG) (52). ETA recommends that the TSH measurements should be taken from women with TAI, those on levothyroxine or starting levothyroxine, women who are undergoing IVF procedure after OS, starting with the second hCG measurement if the woman is pregnant, which is about 6 weeks after the start of stimulation or 3 weeks after ovulation induction. Adjustment of levothyroxine doses is recommended in women who were already receiving treatment before OS, in order to maintain TSH serum values lower than 2.5 mIU/L. It is suggested to treat TAI-positive women with TSH levels above 2.5 and below 4.0 mIU/L or the upper reference limit with a low dose of levothyroxine (usually 25–50 mcg daily) before OS, especially in situations of recurrent miscarriages, in women over 35 years of age, as well as ovarian causes of infertility. Treatment of TAI-positive women with TSH greater than 4.0 mIU/L or above the upper reference limit before OS to maintain TSH measurements below 2.5 mIU/L is recommended, as well as treatment of TAI-negative women with TSH levels above 4, 0 mIU/L or above the upper reference limit before OS (1). The use of a long stimulation protocol with gonadotropin-releasing hormone agonists leads to an increase in the clinical pregnancy rate, namely the use of this protocol

positively correlates with serum estradiol on the day of the final injection and is followed by lower serum TSH values before starting the procedure (53). While some studies have shown that the use of levothyroxine can lead to improved live birth rates and reduced miscarriage rates in women with TAI undergoing IVF (53,54), the results of recent large-scale studies do not support these findings. POSTAL did not show the importance of levothyroxine administration in terms of reducing the rates of miscarriage, clinical pregnancy, and live birth (55). A recent meta-analysis pointed out that the use of levothyroxine does not have a statistically significant effect on the rate of clinical pregnancy, live birth, or premature birth in women with subclinical hypothyroidism and/or TAI in the IVF procedure, but a reduction in the rate of miscarriage is still observed (56). TABLET is another large-scale study that failed to demonstrate the effectiveness of levothyroxine administration in TAI-positive women with the aim of increasing the live birth rate (57). The results of the T4-LIFE study show that, compared with a placebo, levothyroxine treatment did not result in a higher live birth rate in euthyroid anti-TPO At positive women with recurrent miscarriages, based on which the authors do not recommend the routine use of levothyroxine in TPOAb positive women with recurrent miscarriages and normal thyroid function (58).

Conclusion

It seems that day by day we have more and more data on the link between TAI and reproduction and the many consequences this link leads to, recommendations on (non)treatment, the use of ART methods, the selection of a protocol for OS as well as an adequate method of fertilization, the influence of thyroid autoantibodies on oocyte maturation, embryo development, embryo implantation, and on the success rate of the applied method, all with the aim of increasing the live birth rate. However, one should keep in mind a number of cofactors such as age, overweight or obesity, lifestyle habits, and many others that can interfere with conception or pregnancy and, of course, at the same time, if the partner needs to have treatment as well. Nevertheless, we are seeing that, despite everything we know so far, the bidirectional link between TAI and reproduction continues to intrigue with the question of whether thyroid autoantibodies are truly a reflection of a generalized immune response, and not exclusively tissue-specific antibodies, and that we need more fundamental research on their direct effect starting from folliculogenesis to embryogenesis and implantation as well as post-implantation embryo development, but also the composition of the follicular fluid as a microenvironment of enormous importance for oocyte maturation which thyroid autoantibodies reach via the blood-follicle barrier.

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