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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

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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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