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Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction

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BACKGROUND: Thyroid hormone disorders and thyroid peroxidase autoantibodies (TPO-Ab) in women are associated with subfertility and early pregnancy loss. Here, we aim to provide a comprehensive overview of the literature on the pathophysiology of these associations.

METHODS: A review of the literature in the English language was carried out. Relevant studies were identified by searching Medline, EMBASE and the Cochrane Controlled Trials Register from 1975 until March 2014.

RESULTS: From a total of 6108 primary selected articles from the literature search, 105 articles were selected for critical appraisal. Observational data indicate that altered thyroid hormone levels are associated with disturbed folliculogenesis, spermatogenesis, lower fertilization rates and lower embryo quality. Triiodothyronine (T3) in combination with FSH enhances granulosa cell proliferation and inhibits granulosa cell apoptosis by the PI3K/Akt pathway. T3 is considered a biological amplifier of the stimulatory action of gonadotrophins on granulosa cell function. T3 increases the expression of matrix metalloproteinases (MMP), MMP-2, MMP-3, fetal fibronectin and integrin $\alpha5\beta1$T3 in early placental extravillous trophoblasts. Thyroid hormone transporters and receptors are expressed in the ovary, early embryo, endometrium, uterus and placenta. No other data explaining the associations could be retrieved from the literature. The presence of TPO-Ab is negatively associated with spermatogenesis, fertilization and embryo quality, but no data are available on the potential pathophysiological mechanisms.

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CONCLUSIONS: Thyroid hormone disorders and TPO-Ab are associated with disturbed folliculogenesis, spermatogenesis, fertilization and embryogenesis. The pathophysiology of these associations remains largely unknown, as evidence is limited and includes studies using small sample sizes, and often restricted to animal models. There are no studies on the pathophysiology underlying the association between TPO-Ab and reproduction. The available evidence, although limited, supports a role of thyroid hormone in fertility and early pregnancy. This justifies clinical intervention studies on the effects of thyroid hormone supplementation in women with subclinical hypothyroidism and in women prone to develop hypothyroidism due to the presence of TPO-Ab. In addition, more research is needed to identify the underlying mechanisms. This would be of particular interest in women undergoing IVF to pinpoint the effects of thyroid hormone on different parameters of reproduction.

Key words: thyroid hormone / thyroid peroxidase autoantibodies / fertility / embryogenesis / placentation

Introduction

General introduction

Thyroid dysfunction is a common endocrine disorder. In the US National Health and Nutrition Examination Survey (NHANES III), the prevalence of hypothyroidism was 4.6% (0.3 overt and 4.3% subclinical) and the prevalence of hyperthyroidism 1.3% (0.5 overt and 0.7% subclinical) in people without known thyroid disease or a family history of thyroid disease (Hollowell *et al.*, 2002). Thyroid dysfunction is usually acquired and may occur any time in life. In women of reproductive age, the most prevalent cause of thyroid dysfunction is thyroid autoimmunity. Thyroid autoantibodies (Ab) that react with key proteins in the thyroid, such as thyroid peroxidase (TPO) or thyroglobulin (Tg), can induce a chronic lymphocytic thyroiditis that ultimately results in destruction and loss of thyroid function. In Graves' disease, circulating thyroid-stimulating hormone (TSH) receptor autoantibodies can activate the TSH receptor resulting in hyperthyroidism.

Both hypothyroidism and hyperthyroidism have been associated with altered ovarian function, menstrual irregularities, subfertility and higher (recurrent) miscarriage rates (Krassas *et al.*, 2010; van den Boogaard *et al.*, 2011), suggesting that thyroid hormone affects female reproductive organs.

The prevalence of TPO-Ab is 8–14% in women of reproductive age (Krassas *et al.*, 2010). Although the presence of TPO-Ab predisposes to hypothyroidism, the majority of women with TPO-Ab is euthyroid. Importantly, the presence of TPO-Ab combined with normal thyroid function is associated with subfertility, recurrent embryo implantation failure, early pregnancy loss and adverse pregnancy outcomes (Bellver *et al.*, 2008; van den Boogaard *et al.*, 2011). For women with TPO-Ab, no effective treatment intervention is available probably due to lack of knowledge about the underlying pathophysiological mechanisms. Several mechanisms have been proposed for the association between TPO-Ab and subfertility and pregnancy loss. The first hypothesis is that TPO-Ab merely reflects a different level of autoimmunity and that other autoimmune processes cause subfertility or pregnancy loss. The second hypothesis is that the association with subfertility or pregnancy loss is secondary to a subtle deficiency in thyroid hormone. As mentioned above, TPO-Ab can induce a chronic, lymphocytic thyroiditis that results in a lower capacity of the thyroid to adequately adapt to increased demands during pregnancy (Kaprara and Krassas, 2008). The third hypothesis is that the association is confounded by age, because the prevalence of TPO-Ab increases with age and older women face a higher risk of subfertility and miscarriage. The last hypothesis has been rejected by two recent meta-analyses showing that the association between TPO-Ab and subfertility and pregnancy loss is independent of age (Thangaratinam *et al.*, 2011; van den Boogaard *et al.*, 2011).

In this review, we provide a comprehensive overview on the described pathways by which thyroid hormone, and possibly TPO-Ab, influence reproductive biology with special focus on the elements essential to fertilization and early pregnancy.

Regulation of thyroid hormone action

Both thyroid hormone synthesis and thyroid hormone release to the circulation are driven by the pituitary-gland-derived TSH in a classical negative feedback loop. This explains why hypothyroidism in the presence of a functional hypothalamic–pituitary axis results in increased TSH levels while the reverse occurs in hyperthyroidism (Chiamolera and Wondisford, 2009). The human thyroid predominantly produces the biologically inactive prohormone thyroxine (tetraiodotyrosine, T4) and only a small amount of the bioactive hormone triiodothyronine (T3). Less than 0.1% of the total amount of circulating thyroid hormone (T4 and T3) is in the free or unbound form that can be transferred across the plasma membrane into a target cell. It was long thought that thyroid hormones diffuse passively across plasma membranes (Weber *et al.*, 1992; Tagawa *et al.*, 1994; Tagawa and Brown, 2001; Raine and Leatherland, 2003). Currently, we know that thyroid hormone enters the cell by virtue of thyroid hormone transporters, including the monocarboxylate transporters (MCT) 8 and 10 and the solute carrier organic anion transporter family member 1C1 or OATP1C1 (Visser *et al.*, 2008). The intracellular availability of the biologically active thyroid hormone T3 is the net result of a finely tuned system of three distinct iodothyronine deiodinases (DIO1, DIO2, DIO3) with tissue-specific expression that are responsible for thyroid hormone outer ring (type I and II) and inner ring (type I and III) deiodination (Dentice *et al.*, 2013). DIO1 and DIO2 can convert inactive T4 to biologically active T3, whereas both DIO1 and DIO3 are able to inactivate T3. Biologically active T3 finally enters the nucleus and exerts its function through the nuclear thyroid hormone receptors (THR), thyroid hormone receptor alpha (THRA) and beta (THRB) that are expressed in a tissue-specific manner. Since their initial identification, THRs have evolved into central players in a complex system of co-activators and co-repressors (Lazar and Chin, 1990; Oppenheimer, 1999; Brent, 2012).

TPO and TPO-Ab

TPO

TPO is essential for thyroid hormone synthesis *in vivo*. TPO is a glycosylated membrane-bound protein that belongs to the family of mammalian haem peroxidases that have as common denominator a haem prosthetic group acting as an electron acceptor. Other members of this family are dual oxidase 1 (DUOX1), dual oxidase 2 (DUOX2), eosinophil

peroxidase (EPX), lactoperoxidase, myeloperoxidase (MPO), peroxidasin homolog (PXDN), peroxidasin-like protein (PXDNL), prostaglandin G/H synthase 1 (PTGS1) and prostaglandin G/H synthase 2 (PTGS2; Zamocky et al., 2008; Gu et al., 2012). Mammalian haem peroxidases are involved in the catalysis of oxidative reactions, antibacterial processes and inflammation. TPO is highly expressed in the thyroid gland where the protein is located on the apical membrane of thyrocytes. TPO oxidizes iodide to active iodine and links iodinated tyrosine residues to form thyroid hormones, in majority the prohormone T4, but also limited amounts of the biologically active T3 (Miot et al., 2012).

Human TPO-Ab recognizes an immunodominant region comprising overlapping A and B domains on conformationally intact TPO. The amino acids recognized by TPO-Ab are located in the regions with homology to MPO and the complement control protein (McLachlan and Rapoport, 2007). The apical membrane, where TPO is functional, is located on the inner side of the thyroid follicle and is generally not exposed to the human immune system. The generation of TPO-Ab is most likely due to TPO protein or peptides expressed on the basal membrane or a previous phase of destructive thyroid gland damage, exposing the TPO protein localized on the apical membrane to the host-immune system. It is tempting to speculate that although these autoantibodies recognize the TPO protein, they have in fact been raised against one of the other highly homologous members of the MPO family. Indeed, for some patients, cross-reaction of serum TPO-Ab with MPO has been demonstrated. Amino acid sequences recognized by TPO-Ab are also located in regions with homology to MPO (McLachlan and Rapoport, 2007; Davies et al., 2008). This implies that TPO-Ab, as measured with the conventional assay, could have intrinsic affinity to other members of the MPO family that are being expressed in cells involved in reproduction and pregnancy.

Methods

In order to present an up-to-date overview of the effects of thyroid hormone disorders and TPO-Ab on fertility, embryogenesis, implantation and placentation, we searched in Medline, EMBASE and the Cochrane Controlled Trials Register, for relevant studies published from 1975 until March 2014. Relevant research articles published in the English language were obtained and reviewed. Medical subject heading terms used were thyroid hormone, liothyronine, thyroxine, Tg, TPO, thyroid antibody, TPO antibody, Tg antibody, endometrium, placenta, embryo, infertility, fertility, menstrual cycle and spontaneous abortion in relation to thyroid hormones and TPO-Ab. A data limit was specified for the availability of reliable free T4 assays, which precluded articles published before 1975 (Ball et al., 1989). The complete literature search is shown in Supplementary data.

From the publically available RNA microarray studies within the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus, we performed an *in silico* analysis. Data from the *in silico* analysis were used to determine expression of genes related to thyroid hormone metabolism in tissue/cells involved in reproduction (Su et al., 2004; Talbi et al., 2006; Xie et al., 2010).

Results

From a total of 6108 primary selected articles from the literature search, 105 articles were selected for critical appraisal. The results were extracted from studies and were divided in the different target tissues involved in reproduction, namely oocytes, sperm, embryo, endometrium and the placenta. These results are described below.

The results are also summarized in Tables I and II and illustrated in Figs 1 and 2.

Oocytes and ovulation

Thyroid hormone disorders

Ovarian follicles either continue to grow from pre-antral to antral follicles due to survival signals, such as gonadotrophins and growth factors, or undergo atresia. The destiny of the ovarian follicle depends on a subtle balance of expression of hormones and growth factors. In humans, disturbances in thyroid hormone production are responsible for a dysregulation of the hypothalamus–pituitary–gonadal axis, and hypothyroidism is associated with oligomenorrhea (Krassas et al., 2010). In rats, hypothyroidism does not influence the classical pre-ovulatory patterns of LH and

Table I Summary of the available evidence on thyroid hormones and the effect on reproduction.

Thyroid hormones	
Oocytes and ovulation	Thyroid hormone disorders are associated with disturbed folliculogenesis. T3 in combination with FSH enhances granulosa cell proliferation and inhibits granulosa cell apoptosis by the PI3K/Akt pathway. Thyroid hormone transporters and receptors are expressed in the ovary.
Sperm	Hypothyroidism has an adverse effect on human spermatogenesis and negatively affects sperm count and motility as well as morphology. Hyperthyroidism is associated with abnormalities in sperm motility and DNA damage. No studies are available on the mechanisms by which thyroid hormone affects spermatogenesis.
Fertilization and embryogenesis	Hypothyroidism is associated with lower fertilization rates and disturbed embryogenesis. No studies on the pathophysiology have been reported.
Endometrium	Deiodinases, THRA and THRB are expressed in the endometrium Evidence for a direct effect of thyroid hormone on endometrial receptivity or function is lacking.
Implantation	Thyroid hormone stimulates the production of progesterone in granulosa cells and up-regulates LIF. There are no studies on the effect of thyroid hormone on implantation.
Placentation	T3 increases the expression of MMP-2, MMP-3, fetal fibronectin and integrin α5β1T3 in early placental extravillous trophoblasts.

T3, triiodothyronine; THRA, thyroid hormone receptor alpha; THRB, thyroid hormone receptor beta; LIF, leukaemia inhibiting factor; MMP-2,3, matrix metalloproteinase 2,3.

Table II Summary of the available evidence on thyroid peroxidase autoantibodies (TPO-Ab) and the effect on reproduction.

TPO-Antibodies	
Oocytes and ovulation	TPO-Ab are present in follicular fluid. TPO-Ab do not influence the number of retrieved oocytes during controlled ovarian stimulation. There are no studies on a direct effect of TPO-Ab on folliculogenesis.
Sperm	TPO-Ab are more often found in subfertile men compared with a control group. No studies are available that showing a direct effect of TPO-Ab on spermatogenesis
Fertilization and embryogenesis	TPO-Ab are associated with lower fertilization rates and disturbed embryogenesis No literature is available on the pathophysiology.
Endometrium	TPO-Ab do not influence endometrial volume. No studies have been published on direct effects of TPO-Ab on endometrial receptivity or endometrial function.
Implantation	There are no studies on direct effects of TPO-Ab on implantation.
Placentation	TPO-Ab diffuse through the placental barrier There is no evidence for a direct effect of TPO-Ab on early placentation

FSH secretion (Hapon *et al.*, 2010), suggesting that in contrast to humans, hypothyroidism in rats does not have an effect on pituitary gonadotrophin secretion. In domestic cats, no beneficial effect of T4 on *in vitro* antral follicle growth, diameter gain, morphologic development or the amount of viable follicles was found (Wongbandue *et al.*, 2013).

The composition of follicular fluid might be important for developing oocytes and may play a substantial role in oocyte quality. Both T3 and T4 are present in follicular fluid of humans. Both isoforms of THR mRNA are expressed in the human oocyte, and hence thyroid hormone may directly affect the oocyte (Zhang *et al.*, 1997). Conflicting results have been reported on the correlation between serum thyroid hormone levels and follicular fluid levels. One study showed a positive correlation between serum T4 and follicular fluid T4 values (Wakim *et al.*, 1993). In addition, an animal study showed that in follicular fluid T4 levels are generally lower than in blood serum, whereas T3 concentration in follicular fluid is comparable with blood serum levels.

In vitro studies have shown that the growth of rat pre-antral follicles and the levels of ovulated oocytes is stimulated by thyroid hormone. T3 alone is ineffective, but in combination with FSH, it enhances granulosa cell proliferation and inhibits granulosa cell apoptosis by the PI3K/Akt pathway (Jiang *et al.*, 1999, 2000; Zhang *et al.*, 2013). Hypothyroid rats showed similar amounts of corpora lutea, and slightly (although not statistically significant) lower ovulation rates compared with control rats. Hypothyroid rats have higher levels of estrogens, estrogen receptor B (ERβ) and cyp19A1 aromatase expression after ovulation compared with control rats, favouring survival of the corpus luteum (Hapon *et al.*, 2010).

T3 is considered a biological amplifier of the stimulatory action of gonadotrophins on granulosa cell function (Maruo *et al.*, 1991) and all data indicate that thyroid hormone levels seem to play a positive role in follicle development *in vitro* and are important during folliculogenesis and ovulation *in vivo*. Therefore, altered thyroid hormone levels may lead to cyclic irregularities and ovulation disturbances lowering the chance of a successful pregnancy.

In humans, increased expression of THRs was found during follicular growth (Wakim *et al.*, 1995; Zhang *et al.*, 1997). From the publically available RNA microarray studies within the NCBI Gene Expression Omnibus, it appears that thyroid hormone transporters and receptors are expressed—at least to some level—in the ovary (Supplementary data, Fig. S1).

TPO-Ab TPO-Ab were measurable in all samples of follicular fluid obtained from women with thyroid autoimmunity, while they were absent in women without thyroid autoimmunity (Monteleone *et al.*, 2011). The relevance of this observation remains to be elucidated, because it only demonstrates that plasma TPO-Ab can enter follicular fluid. It can however be speculated that thyroid autoantibodies cause a cytotoxic reaction in the follicle fluid leading to damage to the oocyte, which may decrease its quality and development potential. In women with unexplained subfertility and thyroid autoantibodies (TPO-Ab and/or Tg-Ab), the number of oocytes retrieved after ovarian hyperstimulation was not statistically different compared with women without thyroid autoantibodies (Geva *et al.*, 1996; Kilic and Tasdemir, 2008).

In conclusion, thyroid hormone disorders are associated with disturbed folliculogenesis. This is supported by the fact that thyroid hormone transporters and receptors are expressed in the ovary. T3 in combination with FSH enhances granulosa cell proliferation and inhibits granulosa cell apoptosis by the PI3K/Akt pathway. Therefore, altered thyroid hormone levels may lead to cyclic irregularities and ovulation disturbances, thereby lowering the chance of a successful pregnancy.

Although TPO-Ab can be found in follicular fluid, there is no evidence available that it disturbs folliculogenesis.

Sperm

Thyroid hormone disorders

In thyroidectomized prepubertal rats, testosterone levels, number of spermatozoa and sperm motility are decreased (Kumar *et al.*, 1994). In congenitally hypothyroid mice, seminiferous tubules are smaller and contain fewer spermatogonia, spermatocytes, spermatids and spermatozoa compared with controls (Matsushima *et al.*, 1986). Together, these studies indicate that physiological thyroid hormone concentrations are required for normal spermatogenesis in rodents. Male Pax8 null mice are hypothyroid due to thyroid agenesis and have complete azoospermia. However, the azoospermia is the result of a direct morphogenic role of Pax8 in the development of the epididymides and the efferent ducts and not due to congenital hypothyroidism (Wistuba *et al.*, 2007).

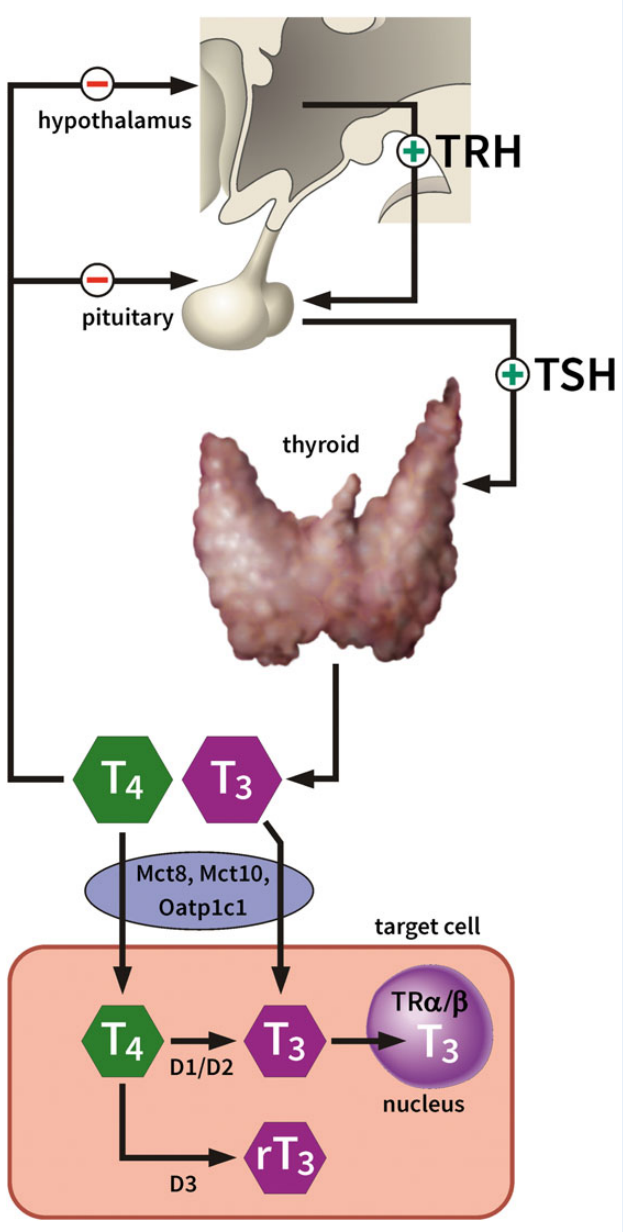

Figure 1 Thyroid hormone physiology. Circulating thyroid hormone concentrations are regulated via a negative feedback system at the level of the hypothalamus and the pituitary. The production of thyroid hormone by the thyroid is regulated by thyroid-stimulating hormone (TSH) produced by the anterior pituitary, which itself is regulated by thyrotropin-releasing hormone (TRH) produced by the hypothalamus. Thyroid hormone circulates as the inactive prohormone thyroxine (T4) and as the active hormone triiodothyronine (T3). Thyroid hormone can only enter target cells by virtue of specific transporters (MCT8, MCT10 and Oatp1c1). In target cells, thyroid hormone can be activated (T4 to T3) or inactivated (T4 to rT3 or T3 to T2) depending on the local activity of specific deiodinases (D1, D2 and D3). Subsequently, active T3 can bind to the nuclear thyroid hormone receptors (TR-alpha and TR-beta) and induce transcription.

Hypothyroidism has an adverse effect on human spermatogenesis and negatively affects sperm count and motility (Rajender *et al.*, 2011) as well as morphology (Corrales Hernandez *et al.*, 1990). Hyperthyroidism is associated with abnormalities in sperm motility (Krassas *et al.*, 2008) and DNA damage (Meeker *et al.*, 2008).

TPO-Ab

The presence of thyroid autoantibodies was higher in subfertile men compared with a control group (Baker *et al.*, 1985).

Altogether, hypothyroidism, hyperthyroidism and presence of TPO-Ab are associated with an adverse effect on sperm parameters. No studies are available showing a causal effect of thyroid hormone and TPO-Ab on sperm parameters. No intervention studies are available on treating thyroid disorders and the effect on sperm parameters. The clinical significance remains therefore unclear, especially since some of the reported abnormalities in sperm parameters do not affect fertility.

Fertilization and embryogenesis

Thyroid hormone disorders

We identified only one study that reports on the effect of thyroid status on fertilization. This study showed that fertilization rates were significantly lower in cows treated with propylthiouracil and who were hypothyroid, compared with control euthyroid cows (Bernal *et al.*, 1999).

In humans, the number of embryos of higher quality was significantly higher in women with subclinical hypothyroidism who were treated with T4 supplementation compared with those who were not. In addition, women that were treated with T4 had a higher live birth rate per initiated cycle, with no difference in the live birth rate in TPO-Ab positive patients (Kim *et al.*, 2011a).

The thyroid hormone transporters and receptors, as well as deiodinases, are expressed in the human pre-implantation embryo (Supplementary data, Fig. S1).

TPO-Ab

Evidence for the influence of TPO-Ab on embryo quality is limited. One study showed a decreased percentage of 3–4 cell stage mouse embryos cultured in serum with TPO-Ab compared with mouse embryos cultured in normal mouse serum (74 versus 90%, $P < 0.05$) but the number of expanded blastocysts (66 versus 73%) and hatching blastocysts (36 versus 37%) did not significantly differ between the two groups (Lee *et al.*, 2009).

In a study of 14 women with TPO-Ab (also in follicular fluid), oocyte fertilization, grade A embryos and pregnancy rates were lower compared with 17 women without TPO-Ab and this effect was independent of thyroid hormone status (Monteleone *et al.*, 2011). Another study found no statistical differences in the number of grade 1 and grade 2 embryos comparing women with unexplained subfertility and positive thyroid autoantibodies (TPO-Ab and/or Tg-Ab) with unexplained subfertility without thyroid autoantibodies (Kilic and Tasdemir, 2008). This discrepancy might be due to the fact that this study also included women positive for Tg-Ab.

In conclusion, both hypothyroidism and the presence of TPO-Ab seem to negatively affect fertilization rates and embryo quality, but to date, only associations have been reported and studies exploring the pathophysiology are lacking.

Endometrium

Thyroid hormone disorders

There is ample evidence that DIO2 and DIO3 are present in human endometrium throughout the menstrual cycle (Kohrle, 1999; Aghajanova *et al.*, 2009, 2011). The expression in the mid-secretory phase is lower and the

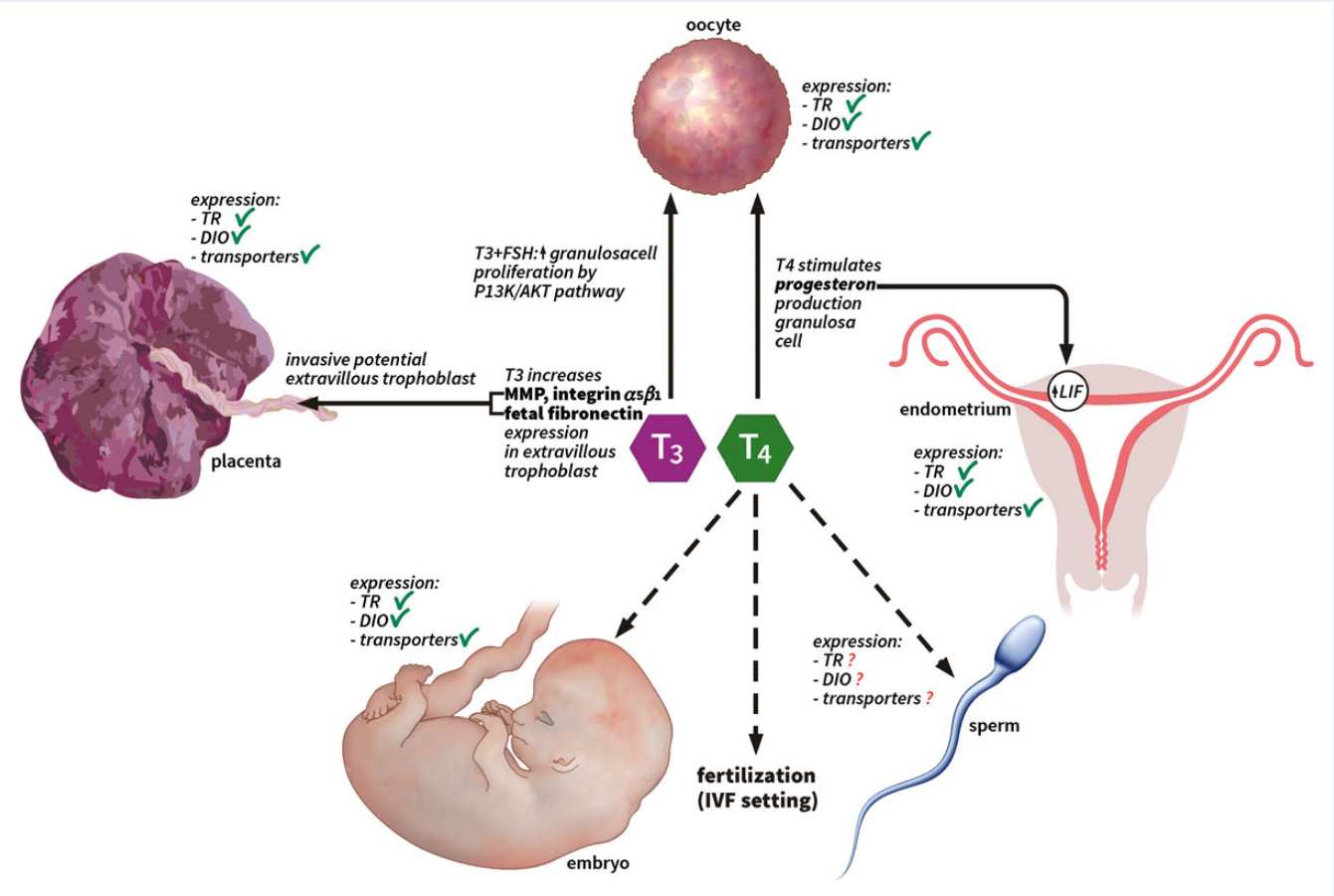

Figure 2 Mechanisms of action of thyroid hormones in the reproductive system. Schematic summary of known effects and/or associations of thyroid hormone and the reproductive system. Solid lines indicate an effect of T4 administration. Dotted lines indicate associations without evidence for causality. For each tissue/cell-type expression of TR, deiodinases (DIO) and thyroid hormone transporters is indicated. Thyroid peroxidase autoantibody (TPO-Ab) is not shown because a lack of evidence for a causal relationship between TPO-Ab and function of the reproductive system. MMP, metalloproteinases.

cyclic changes of deiodinase activities show an inverse relationship with progesterone levels (Catalano *et al.*, 2007; Aghajanova *et al.*, 2011). THRA and THRB are expressed in the glandular endometrium with a peak during the mid-secretory phase. The expression of deiodinases, THRA and THRB in the endometrium indicates a dynamic local regulation of bioavailable thyroid hormone metabolites.

Evaluation of publically available expression data in the NCBI Gene Expression Omnibus supports these data and also demonstrates the presence of thyroid hormone transporters and even relatively high expression of co-activators and repressors of the THRs in the endometrium and uterus (Supplementary data, Fig. S1).

TPO-Ab

Endometrial volume is an important parameter to evaluate endometrial receptivity and therefore a possible predictor for successful implantation (Coulam *et al.*, 1994; Noyes *et al.*, 1995). In euthyroid women with unexplained infertility, there was no difference in endometrial volume between subjects positive or negative for TPO-Ab, whereas the pregnancy rate after IVF was lower in the TPO-Ab positive group (Kilic and Tasdemir, 2008).

Analysis of mRNA expression data from the non-pregnant human uterus (Samborski *et al.*, 2013; Supplementary data, Table SI) shows that the MPO domain-encoding genes PXDN and PTGS2 have relatively high expression levels compared with TPO and the other MPO domain-encoding genes in this tissue. PTGS2-mediated prostaglandin synthesis in mouse is known to be essential for ovulation, fertilization, implantation and decidualization (Lim *et al.*, 1997). The function of PXDN homologue in the endometrium is unknown.

In conclusion, deiodinases, THRA and THRB are expressed in the endometrium suggesting a functional role for thyroid hormone, but there are no studies available that demonstrate a direct effect on endometrial receptivity or endometrial function. TPO mRNA is expressed in the endometrium at a relatively low level and protein expression has never been demonstrated, which makes a direct pathophysiological effect of TPO-Ab on TPO in the endometrium unlikely.

Implantation

Thyroid hormone disorders

In this review, implantation is defined as the direct contact between the maternal and fetal interface prior to the invasion of extravillous trophoblasts into the maternal spiral arteries. One study showed that T4 increases progesterone production in human granulosa cells *in vitro* when administered in combination with insulin and gonadotrophins (Wakim *et al.*, 1995). As progesterone is responsible for building up

the endometrial lining for an optimal implantation and for decreasing the maternal immune response to allow for the acceptance of the pregnancy, T4 bioavailability may have a mediating role in this process.

Leukaemia inhibitory factor (LIF) is involved in the embryo implantation process and expressed in the mid-secretory endometrium (Koot et al., 2012). TSH significantly up-regulated LIF expression in endometrial cell cultures, suggesting a potential role of TSH in the implantation process (Aghajanova et al., 2011).

TPO-Ab

Analysis of mRNA expression data from non-pregnant human uterus (Samborski et al., 2013; Supplementary data, Table SI) shows that the MPO domain-encoding genes PXDN and PTGS2 have relatively high expression levels in this tissue. In humans, the effect of PTGS2 is less clear than in mice, but PTGS2 is known to play a role in female fertility (Cha et al., 2012). Mice where PGTS2 expression was limited were infertile or produced small litters or no litters. A cohort study of 34 women showed that prostaglandin synthesis appears to be disrupted in patients with repeated IVF failure compared with fertile controls (Achache et al., 2010). This suggests that reduced prostaglandin synthesis in the human endometrium may lead to poor endometrial receptivity. The function of PXDN homologue in the endometrium is unknown. The possibility that TPO-Ab might be able to recognize PTGS2 and that PTGS2 is important for an ongoing pregnancy in human and mice and makes it tempting to speculate that there might be an effect of TPO-Ab on PTGS2. No data supporting this speculation are available.

In conclusion, thyroid hormone stimulates the production of progesterone in granulosa cells and up-regulates LIF expression. Both are important for the implantation process. No evidence is available on a direct pathophysiological effect of thyroid hormones or TPO-Ab on implantation.

Placentation

Thyroid hormone disorders

The placenta is responsible for the exchange of oxygen, nutrients, hormones and growth factors and their waste products between mother and fetus. The migration of extravillous trophoblasts into the maternal uterine spiral arteries allows increased blood flow to the placenta. Cell adhesion molecules (Damsky et al., 1993), metalloproteinases (MMP-2 and MMP-3), tissue inhibitors of metalloproteinases, fibronectin and integrin $\alpha5\beta1$ are important for the invasion process (Sato et al., 1994; Aplin et al., 1999). T3 is known to increase the expression of MMP-2, MMP-3, fetal fibronectin and integrin $\alpha5\beta1$T3 in cultured early (8–12 weeks) placental extravillous trophoblasts, suggesting that thyroid hormone plays a vital role regulating the invasive potential of extravillous trophoblasts (Oki, 2004).

One of the effects of thyroid hormone is the stimulation of the placental secretion of progesterone and human placental lactogen. Progesterone is essential for the endometrial lining and an optimal nidation, as well as inducing the local immune tolerance that decreases the maternal immune response and prevents rejection of the fetal allograft (Maruo et al., 1991). Human placental lactogen increases the fetal glucose supply by decreasing maternal fatty acids stores through altering maternal insulin secretion. T3 through the THRB stimulates the expression and release of placental lactogen in cultured human trophoblasts (Stephanou and Handwerger, 1995). T4 both increases vascular endothelial growth factor in trophoblasts as well as the height of the trophoblast epithelium in gilts (Souza et al., 2011).

Thyroid hormone metabolism in the placenta seems tightly regulated. All three types of deiodinase are expressed in placenta (Koopdonk-Kool et al., 1996), and the relatively high levels of DIO3 expression limit the transfer of maternal circulating thyroid hormones to the fetus (Mortimer et al., 1996). The placenta is responsive to T3 and contains thyroid receptors not only at term, but also during early gestation (Mukku et al., 1983; Maruo et al., 1992; Laoag-Fernandez et al., 2004). High affinity-specific T3-binding proteins are present in the trophoblast membrane and are responsible for uptake of T3 by trophoblast cells (Nishii et al., 1989; Pontecorvi and Robbins, 1989; Mitchell et al., 1992). All these *in vitro* data were confirmed by our *in silico* analysis. There is a relatively high placental expression of all factors involved in thyroid hormone action (Supplementary data, Fig. S1).

TPO-Ab

Although TPO-Ab diffuse through the placental barrier during the third trimester of pregnancy (Seror et al., 2014), there is no evidence that this is also true in early pregnancy. Since the characteristics of the placental barrier change only slightly after the first trimester, transfer of TPO-Ab at all stages of pregnancy seems likely. A recent cohort study supported that the increased risk of TPO-Ab positive women on adverse pregnancy outcomes was independent of thyroid function (Korevaar et al., 2013). Possibly, there are direct targets for TPO-Ab, other than TPO, at the maternal–fetal interface that affect placentation and ongoing pregnancy. In particular, expression of the MPO-domain-containing protein peroxidasin homologue is very abundant in both trophoblasts and decidua (www.proteinatlas.org and Supplementary data, Table SI).

It is important to bear in mind that TPO-Ab are also associated with the presence of other autoantibodies, such as zona pellucida autoantibodies. Zona pellucida and thyroid tissue seem to share some antigens and might cross react. It has been postulated that the zona pellucida may be the target of TPO-Ab (Kelkar et al., 2005). MPO is involved in the catalysis of oxidative reactions, antibacterial processes and inflammation, which hypothetically may lead to an increased immune response. It is also hypothesized that TPO-Ab reflect a general immune response, resulting in subfertility and complications during early pregnancy. Kim et al. (2011b) showed that tumour necrosis factor alpha and interleukin-10-expressing CD3/CD4 cell ratios and non-organ-specific antibodies were significantly increased in women with thyroid autoantibodies. They additionally concluded that women suffering from recurrent miscarriage with thyroid autoantibodies have significantly elevated serum levels of natural killer cells (Kim et al., 2011b). No correlation could be established between the presence of TPO-Ab and uterine-natural killer cells in women suffering for recurrent pregnancy loss after IVF and their levels of TPO-Ab (Mariee et al., 2012).

In conclusion, T3 increases the expression of MMP-2, MMP-3, fetal fibronectin and integrin $\alpha5\beta1$ in early placental extravillous trophoblasts, suggesting that thyroid hormone plays a vital role in the regulation of the invasive potential of extravillous trophoblasts. TPO-Ab diffuse through the placental barrier but there is no evidence published that TPO-Ab directly affect placentation.

Discussion

An association exists between thyroid hormone disturbances and/or TPO-Ab and subfertility and early pregnancy loss, but the exact pathophysiology is unknown (van den Boogaard et al., 2011).

This review shows that altered thyroid hormone levels are associated with disturbed folliculogenesis and spermatogenesis, lower fertilization rates and lower embryo quality.

Thyroid hormone levels seem to play a positive role for ovulation and folliculogenesis. T3 in combination with FSH enhances granulosa cell proliferation and inhibits granulosa cell apoptosis by the PI3K/Akt pathway (Jiang *et al.*, 1999, 2000; Zhang *et al.*, 2013). Hypothyroid rats have higher levels of estrogens, ERβ and cyp19A1 aromatase expression after ovulation compared with control rats, favouring survival of the corpus luteum (Hapon *et al.*, 2010).

T3 is considered a biological amplifier of the stimulatory action of gonadotrophins on granulosa cell function (Maruo *et al.*, 1991). Thyroid hormone levels seem to play a positive role for embryo quality, because treatment with T4 in women with subclinical hypothyroidism resulted in a higher embryo quality (Bernal *et al.*, 1999).

T3 is known to increase the expression of MMP-2, MMP-3, fetal fibronectin and integrin α5β1 in cultured early (8–12 weeks) placental extravillous trophoblasts, suggesting that thyroid hormone plays a vital role regulating the invasive potential of extravillous trophoblasts (Oki, 2004).

Thyroid hormone transporters, receptors and their associated proteins are expressed in the ovary, the early embryo (Xie *et al.*, 2010), endometrium (Talbi *et al.*, 2006), uterus and placenta (Su *et al.*, 2004; Supplementary data, Fig. S1). No other data explaining the associations could be retrieved from the literature and the underlying mechanism for these clinical parameters remains unclear.

The available evidence, although limited, supports a role for thyroid hormone in reproduction and early pregnancy. The fact that almost all factors essential for thyroid hormone action, such as THRA and THRB, thyroid hormone transporters and deiodinases, are expressed in several tissues involved in reproduction, namely ovary, endometrium, uterus and placenta, indicates a dynamic local regulation of bioavailable thyroid hormone metabolites. It is important to realize that thyroid hormone and associated proteins are expressed in multiple tissues, other than the reproductive organs, but that the expression levels seems not that high. It is unknown if the relatively low expression levels also mean that there is a functional effect of these factors. Expression of these factors *per se* does not explain a possible direct pathway.

The presence of TPO-Ab negatively influences folliculogenesis, spermatogenesis, fertilization rates, embryo quality and pregnancy rates, but no data are available on the potential mechanisms. Low-to-absent expression of TPO mRNA expression in the endometrium, uterus and placenta makes a direct effect of TPO-Ab unlikely (Supplementary data, Table SI).

TPO expression is low or absent in the endometrium and placenta, especially compared with other peroxidases. It is still very interesting to speculate that other peroxidases, such as PXDN or PTGS, are the target for TPO-Ab and cause an increased immunological response. There is however no evidence showing that TPO-Ab have binding affinity for these other peroxidases. Studies on a possible increased immunological response in women with TPO-Ab are very limited.

Summary and clinical relevance

The available evidence, although limited, supports a role of thyroid hormone in fertility and early pregnancy. The hypothesis that the associated subfertility or pregnancy loss is secondary to a subtle deficiency in thyroid hormone concentrations is therefore more likely than a direct pathogenic effect of TPO-Ab. Future research should be focusing on thyroid hormone disturbances and their clinical and pathophysiological effects. Although understanding the molecular signalling of thyroid hormone is very interesting, a clinical intervention study is more pragmatic to investigate whether thyroid hormone supplementation improves fertility or early pregnancy outcomes in women with subclinical hypothyroidism and in women prone to develop hypothyroidism due to the presence of TPO-Ab. There is a need for clinical studies given the worldwide discussion on treating pregnant women with subclinical thyroid dysfunction. There is a very broad variation in the treatment and screening of pregnant women for thyroid disorders in pregnancy (Vaidya *et al.*, 2012). Guidelines provide different advice regarding when to screen or treat pregnant women with thyroid hormone supplementation (De *et al.*, 2012). Currently, two studies are recruiting women with TPO-Ab and a history of (recurrent) miscarriage to investigate if treatment with levothyroxine improves live birth rates, the T4-LIFE study (NTR 3364) and the TABLET Study (ISRCTN15948785). Randomized studies are needed to study the effect of treating subclinical hypothyroidism in pregnancy. This would also be of particular interest in women with subclinical hypothyroidism and/or TPO-Ab undergoing IVF to pinpoint specific effects of thyroid hormone on reproduction. Valuable data on parameters such as number of follicles, number of oocytes, fertilization rates, embryo quality, implantation rates, and pregnancy outcome could be obtained and may lead to approaches to improve the fertility and pregnancy outcomes, and at the same time provide clues on where to start more fundamental studies on the underlying pathophysiological mechanisms.

Supplementary data

Supplementary data is available at *http://humupd.oxfordjournals.org/* online.

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Authors' roles

C.R.S., E.F., S.M., M.G. and P.H.B. all contributed substantially to the conception and design of this review. R.V. and V.M. performed the literature search, screened all titles, abstracts and articles and extracted data. G.A. was responsible for the *in silico* analysis and gene expression data. C.R.S. and G.A. drafted the introduction of the article. M.G. drafted the subchapter sperm and the subchapter fertilization and embryogenesis. V.M. drafted the subchapters oocyte and ovulation and the subchapter implantation. R.V. drafted the other sections of the article. All other authors critically revised multiple versions of the manuscript. All authors gave their final approval of the version to be published.

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Conflict of interest

None declared.

References

Achache H, Tsafrir A, Prus D, Reich R, Revel A. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing *in vitro* fertilization. *Fertil Steril* 2010;**94**:1271–1278.

Aghajanova L, Lindeberg M, Carlsson IB, Stavreus-Evers A, Zhang P, Scott JE, Hovatta O, Skjoldebrand-Sparre L. Receptors for thyroid-stimulating hormone and thyroid hormones in human ovarian tissue. *Reprod Biomed Online*2009; **18**:337–347.

Aghajanova L, Stavreus-Evers A, Lindeberg M, Landgren BM, Sparre LS, Hovatta O. Thyroid-stimulating hormone receptor and thyroid hormone receptors are involved in human endometrial physiology. *Fertil Steril* 2011;**95**:230–237.

Aplin JD, Haigh T, Jones CJ, Church HJ, Vicovac L. Development of cytotrophoblast columns from explanted first-trimester human placental villi: role of fibronectin and integrin alpha5beta1. *Biol Reprod* 1999;**60**:828–838.

Baker HW, Clarke GN, McGowan MP, Koh SH, Cauchi MN. Increased frequency of autoantibodies in men with sperm antibodies. *Fertil Steril* 1985;**43**:438–441.

Ball R, Freedman DB, Holmes JC, Midgley JE, Sheehan CP. Low-normal concentrations of free thyroxin in serum in late pregnancy: physiological fact, not technical artefact. *Clin Chem* 1989;**35**:1891–1896.

Bellver J, Soares SR, Alvarez C, Munoz E, Ramirez A, Rubio C, Serra V, Remohi J, Pellicer A. The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. *Hum Reprod* 2008;**23**:278–284.

Bernal A, DeMoraes GV, Thrift TA, Willard CC, Randel RD. Effects of induced hypothyroidism on ovarian response to superovulation in Brahman (*Bos indicus*) cows. *J Anim Sci* 1999;**77**:2749–2756.

Brent GA. Mechanisms of thyroid hormone action. *J Clin Invest* 2012;**122**:3035–3043.

Catalano RD, Critchley HO, Heikinheimo O, Baird DT, Hapangama D, Sherwin JR, Charnock-Jones DS, Smith SK, Sharkey AM. Mifepristone induced progesterone withdrawal reveals novel regulatory pathways in human endometrium. *Mol Hum Reprod* 2007;**13**:641–654.

Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy. *Nat Med* 2012;**18**:1754–1767.

Chiamolera MI, Wondisford FE. Minireview: thyrotropin-releasing hormone and the thyroid hormone feedback mechanism. *Endocrinology* 2009;**150**:1091–1096.

Corrales Hernandez JJ, Miralles Garcia JM, Garcia Diez LC. Primary hypothyroidism and human spermatogenesis. *Arch Androl* 1990;**25**:21–27.

Coulam CB, Bustillo M, Soenksen DM, Britten S. Ultrasonographic predictors of implantation after assisted reproduction. *Fertil Steril* 1994;**62**:1004–1010.

Damsky C, Sutherland A, Fisher S. Extracellular matrix 5: adhesive interactions in early mammalian embryogenesis, implantation, and placentation. *FASEB J* 1993;**7**:1320–1329.

Davies MJ, Hawkins CL, Pattison DI, Rees MD. Mammalian heme peroxidases: from molecular mechanisms to health implications. *Antioxid Redox Signal* 2008;**10**:1199–1234.

De GL, Abalovich M, Alexander EK, Amino N, Barbour L, Cobin RH, Eastman CJ, Lazarus JH, Luton D, Mandel SJ *et al*. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2012;**97**:2543–2565.

Dentice M, Marsili A, Zavacki A, Larsen PR, Salvatore D. The deiodinases and the control of intracellular thyroid hormone signaling during cellular differentiation. *Biochim Biophys Acta* 2013;**1830**:3937–3945.

Geva E, Vardinon N, Lessing JB, Lerner-Geva L, Azem F, Yovel I, Burke M, Yust I, Grunfeld R, Amit A. Organ-specific autoantibodies are possible markers for reproductive failure: a prospective study in an *in vitro* fertilization-embryo transfer programme. *Hum Reprod* 1996;**11**:1627–1631.

Gu SQ, Bakthavachalu B, Han J, Patil DP, Otsuka Y, Guda C, Schoenberg DR. Identification of the human PMR1 mRNA endonuclease as an alternatively processed product of the gene for peroxidasin-like protein. *RNA* 2012; **18**:1186–1196.

Hapon MB, Gamarra-Luques C, Jahn GA. Short term hypothyroidism affects ovarian function in the cycling rat. *Reprod Biol Endocrinol* 2010;**8**:14.

Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* 2002;**87**:489–499.

Jiang JY, Miyoshi K, Umezu M, Sato E. Superovulation of immature hypothyroid rdw rats by thyroxine therapy and the development of eggs after in vitro fertilization. *J Reprod Fertil* 1999;**116**:19–24.

Jiang JY, Umezu M, Sato E. Improvement of follicular development rather than gonadotrophin secretion by thyroxine treatment in infertile immature hypothyroid rdw rats. *J Reprod Fertil* 2000;**119**:193–199.

Kaprara A, Krassas GE. Thyroid autoimmunity and miscarriage. *Hormones (Athens)* 2008;**7**:294–302.

Kelkar RL, Meherji PK, Kadam SS, Gupta SK, Nandedkar TD. Circulating auto-antibodies against the zona pellucida and thyroid microsomal antigen in women with premature ovarian failure. *J Reprod Immunol* 2005;**66**:53–67.

Kilic S, Tasdemir N. The effect of anti-thyroid antibodies on endometrial volume, embryo grade and IVF outcome. *Gynecol Endocrinol* 2008;**24**:649–655.

Kim CH, Ahn JW, Kang SP, Kim SH, Chae HD, Kang BM. Effect of levothyroxine treatment on in vitro fertilization and pregnancy outcome in infertile women with subclinical hypothyroidism undergoing in vitro fertilization/intracytoplasmic sperm injection. *Fertil Steril* 2011a;**95**:1650–1654.

Kim NY, Cho HJ, Kim HY, Yang KM, Ahn HK, Thornton S, Park JC, Beaman K, Gilman-Sachs A, Kwak-Kim J. Thyroid autoimmunity and its association with cellular and humoral immunity in women with reproductive failures. *Am J Reprod Immunol* 2011b;**65**:78–87.

Kohrle J. Local activation and inactivation of thyroid hormones: the deiodinase family. *Mol Cell Endocrinol* 1999;**151**:103–119.

Koopdonk-Kool JM, de Vijlder JJ, Veenboer GJ, Ris-Stalpers C, Kok JH, Vulsma T, Boer K, Visser TJ. Type II and type III deiodinase activity in human placenta as a function of gestational age. *J Clin Endocrinol Metab* 1996;**81**:2154–2158.

Koot YE, Teklenburg G, Salker MS, Brosens JJ, Macklon NS. Molecular aspects of implantation failure. *Biochim Biophys Acta* 2012;**1822**:1943–1950.

Korevaar TI, Schalekamp-Timmermans S, de Rijke YB, Visser WE, Visser W, de Muinck Keizer-Schrama SM, Hofman A, Ross HA, Hooijkaas H, Tiemeier H *et al*. Hypothyroxinemia and TPO-antibody positivity are risk factors for premature delivery: the generation R study. *J Clin Endocrinol Metab* 2013;**98**:4382–4390.

Krassas GE, Papadopoulou F, Tziomalos K, Zeginiadou T, Pontikides N. Hypothyroidism has an adverse effect on human spermatogenesis: a prospective, controlled study. *Thyroid* 2008;**18**:1255–1259.

Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. *Endocr Rev* 2010;**31**:702–755.

Kumar PN, Aruldhas MM, Juneja SC. Influence of hypothyroidism induced at prepuberty on epididymal lipids and the number and motility of spermatozoa in rats. *Int J Androl* 1994;**17**:262–270.

Laoag-Fernandez JB, Matsuo H, Murakoshi H, Hamada AL, Tsang BK, Maruo T. 3,5,3′-Triiodothyronine down-regulates Fas and Fas ligand expression and suppresses caspase-3 and poly (adenosine 5′-diphosphate-ribose) polymerase cleavage and apoptosis in early placental extravillous trophoblasts in vitro. *J Clin Endocrinol Metab* 2004;**89**:4069–4077.

Lazar MA, Chin WW. Nuclear thyroid hormone receptors. *J Clin Invest* 1990; **86**:1777–1782.

Lee YL, Ng HP, Lau KS, Liu WM, WS O, Yeung WS, 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;**91**:2104–2109.

Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 1997; **91**:197–208.

Mariee NG, Tuckerman E, Laird S, Li TC. The correlation of autoantibodies and uNK cells in women with reproductive failure. *J Reprod Immunol* 2012;**95**:59–66.

Maruo T, Matsuo H, Mochizuki M. Thyroid hormone as a biological amplifier of differentiated trophoblast function in early pregnancy. *Acta Endocrinol (Copenh)* 1991;**125**:58–66.

Maruo T, Hiramatsu S, Otani T, Hayashi M, Mochizuki M. Increase in the expression of thyroid hormone receptors in porcine granulosa cells early in follicular maturation. *Acta Endocrinol (Copenh)* 1992;**127**:152–160.

Matsushima M, Kuroda K, Shirai M, Ando K, Sugisaki T, Noguchi T. Spermatogenesis in Snell dwarf, little and congenitally hypothyroid mice. *Int J Androl* 1986;**9**:132–140.

McLachlan SM, Rapoport B. Thyroid peroxidase as an autoantigen. *Thyroid* 2007; **17**:939–948.

Meeker JD, Singh NP, Hauser R. Serum concentrations of estradiol and free T4 are inversely correlated with sperm DNA damage in men from an infertility clinic. *J Androl* 2008;**29**:379–388.

Miot F, Dupuy C, Dumont J, Rousset B. Thyroid hormone synthesis and secretion. 2012. Retrieved from http://www.thyroidmanager.org/.

Mitchell AM, Manley SW, Mortimer RH. Uptake of L-tri-iodothyronine by human cultured trophoblast cells. *J Endocrinol* 1992;**133**:483–486.

Monteleone P, Parrini D, Faviana P, Carletti E, Casarosa E, Uccelli A, Cela V, Genazzani AR, Artini PG. Female infertility related to thyroid autoimmunity: the ovarian follicle hypothesis. *Am J Reprod Immunol* 2011;**66**:108–114.

Mortimer RH, Galligan JP, Cannell GR, Addison RS, Roberts MS. Maternal to fetal thyroxine transmission in the human term placenta is limited by inner ring deiodination. *J Clin Endocrinol Metab* 1996;**81**:2247–2249.

Mukku VR, Kirkland JL, Hardy M, Stancel GM. Evidence for thyroid hormone receptors in uterine nuclei. *Metabolism* 1983;**32**:142–145.

Nishii H, Ashitaka Y, Maruo M, Mochizuki M. Studies on the nuclear 3, 5, 3′-triiodo-L-thyronine binding sites in cytotrophoblast. *Endocrinol Jpn* 1989; **36**:891–898.

Noyes N, Liu HC, Sultan K, Schattman G, Rosenwaks Z. Endometrial thickness appears to be a significant factor in embryo implantation in in-vitro fertilization. *Hum Reprod* 1995;**10**:919–922.

Oki NM. Effects of 3,5,3′-triiodothyronine on the invasive potential and the expression of integrins and matrix metalloproteinases in cultured early placental extravillous trophoblasts. *J Clin Endocrinol Metab* 2004;**89**:5213–5221.

Oppenheimer JH. Evolving concepts of thyroid hormone action. *Biochimie* 1999; **81**:539–543.

Pontecorvi A, Robbins J. The plasma membrane and thyroid hormone entry into cells. *Trends Endocrinol Metab* 1989;**1**:90–94.

Raine JC, Leatherland JF. Trafficking of L-triiodothyronine between ovarian fluid and oocytes of rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol B Biochem Mol Biol* 2003;**136**:267–274.

Rajender S, Monica MG, Walter L, Agarwal A. Thyroid, spermatogenesis, and male infertility. *Front Biosci (Elite Ed)* 2011;**3**:843–855.

Samborski A, Graf A, Krebs S, Kessler B, Reichenbach M, Reichenbach HD, Ulbrich SE, Bauersachs S. Transcriptome changes in the porcine endometrium during the preattachment phase. *Biol Reprod* 2013;**89**:134.

Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M. A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* 1994; **370**:61–65.

Seror J, Amand G, Guibourdenche J, Ceccaldi PF, Luton D. Anti-TPO antibodies diffusion through the placental barrier during pregnancy. *PLoS One* 2014; **9**:e84647.

Souza CA, Ocarino NM, Silva JF, Boeloni JN, Nascimento EF, Silva IJ, Castro RD, Moreira LP, Almeida FR, Chiarini-Garcia H *et al.* Administration of thyroxine affects the morphometric parameters and VEGF expression in the uterus and placenta and the uterine vascularization but does not affect reproductive parameters in gilts during early gestation. *Reprod Domest Anim* 2011;**46**:e7–16.

Stephanou A, Handwerger S. Retinoic acid and thyroid hormone regulate placental lactogen expression in human trophoblast cells. *Endocrinology* 1995;**136**:933–938.

Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G *et al.* A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA* 2004;**101**:6062–6067.

Tagawa M, Brown CL. Entry of thyroid hormones into tilapia oocytes. *Comp Biochem Physiol B Biochem Mol Biol* 2001;**129**:605–611.

Tagawa M, Ogasawara T, Sakamoto T, Miura T, Yamauchi K, Hirano T. Thyroid hormone concentrations in the gonads of wild chum salmon during maturation. *Fish Physiol Biochem* 1994;**13**:233–240.

Talbi S, Hamilton AE, Vo KC, Tulac S, Overgaard MT, Dosiou C, Le SN, Nezhat CN, Kempson R, Lessey BA *et al.* Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology* 2006;**147**:1097–1121.

Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J, Coomarasamy A. Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. *BMJ* 2011;**342**:d2616.

Vaidya B, Hubalewska-Dydejczyk A, Laurberg P, Negro R, Vermiglio F, Poppe K. Treatment and screening of hypothyroidism in pregnancy: results of a European survey. *Eur J Endocrinol* 2012;**166**:49–54.

van den Boogaard E, Vissenberg R, Land JA, van Wely M, van der Post JA, Goddijn M, 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.

Visser WE, Friesema EC, Jansen J, Visser TJ. Thyroid hormone transport in and out of cells. *Trends Endocrinol Metab* 2008;**19**:50–56.

Wakim AN, Polizotto SL, Buffo MJ, Marrero MA, Burholt DR. Thyroid hormones in human follicular fluid and thyroid hormone receptors in human granulosa cells. *Fertil Steril* 1993;**59**:1187–1190.

Wakim AN, Polizotto SL, Burholt DR. Augmentation by thyroxine of human granulosa cell gonadotrophin-induced steroidogenesis. *Hum Reprod* 1995; **10**:2845–2848.

Weber GM, Okimoto DK, Richman NH III, Grau EG. Patterns of thyroxine and triiodothyronine in serum and follicle-bound oocytes of the tilapia, *Oreochromis mossambicus*, during oogenesis. *Gen Comp Endocrinol* 1992;**85**:392–404.

Wistuba J, Mittag J, Luetjens CM, Cooper TG, Yeung CH, Nieschlag E, Bauer K. Male congenital hypothyroid $Pax8^{-/-}$ mice are infertile despite adequate treatment with thyroid hormone. *J Endocrinol* 2007;**192**:99–109.

Wongbandue G, Jewgenow K, Chatdarong K. Effects of thyroxin (T4) and activin A on in vitro growth of preantral follicles in domestic cats. *Theriogenology* 2013; **79**:824–832.

Xie D, Chen CC, Ptaszek LM, Xiao S, Cao X, Fang F, Ng HH, Lewin HA, Cowan C, Zhong S. Rewirable gene regulatory networks in the preimplantation embryonic development of three mammalian species. *Genome Res* 2010;**20**:804–815.

Zamocky M, Jakopitsch C, Furtmuller PG, Dunand C, Obinger C. The peroxidase-cyclooxygenase superfamily: reconstructed evolution of critical enzymes of the innate immune system. *Proteins* 2008;**72**:589–605.

Zhang SS, Carrillo AJ, Darling DS. Expression of multiple thyroid hormone receptor mRNAs in human oocytes, cumulus cells, and granulosa cells. *Mol Hum Reprod* 1997;**3**:555–562.

Zhang C, Guo L, Zhu B, Feng Y, Yu S, An N, Wang X. Effects of 3, 5, 3′-triiodothyronine (t3) and follicle stimulating hormone on apoptosis and proliferation of rat ovarian granulosa cells. *Chin J Physiol* 2013;**56**:298–305.