Gynecologic and Obstetric Investigation

Gynecol Obstet Invest , DOI: 10.1159/000539265
Received: March 5, 2024
Accepted: May 3, 2024
Published online: May 8, 2024

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ISSN: 0378-7346 (Print), eISSN: 1423-002X (Online)
https://www.karger.com/GOI
Gynecologic and Obstetric Investigation

Research Article

Coexistence of endometriosis and thyroid autoimmunity in infertile women: impact on in-vitro fertilization and reproductive outcomes.

Sara **Korošec** [1], Gaetano **Riemma** [2], Vesna **Šalamun** [1], Anita **Franko Rutar** [3], Antonio Simone **Laganà** [4,5], Vito **Chiantera** [5,6], Pasquale **De Franciscis** [2], Helena **Ban Frangež** [1]

Running Title: Reproductive outcomes in women with endometriosis and thyroid autoimmunity

[1] *Department of Human Reproduction, Division of Gynaecology and Obstetrics, University Medical Centre Ljubljana, 1000 Ljubljana, Slovenia.*
[2] *Obstetrics and Gynecology Unit, Department of Woman, Child and General and Specialized Surgery, University of Campania "Luigi Vanvitelli"; 80128 Naples, Italy.*
[3] *Zdravstveni dom Trebnje (Health center Trebnje), 8210 Trebnje, Slovenia.*
[4] *Unit of Obstetrics and Gynecology, "Paolo Giaccone" Hospital, 90127 Palermo, Italy*
[5] *Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, 90127 Palermo, Italy.*
[6] *Unit of Gynecologic Oncology, National Cancer Institute - IRCCS - Fondazione "G. Pascale", 80131 Naples, Italy.*

Corresponding author:
Helena Ban Frangež MD, PhD
Department of Human Reproduction, Division of Gynaecology and Obstetrics, University Medical Centre Ljubljana, 1000 Ljubljana, Slovenia;
Šlajmerjeva ulica 3, 1000 Ljubljana
E-mail: helena.ban@kclj.si;
Phone: (+386) 41 336 441

Number of Tables: 5
Number of Figures: 2
Word count: 3844

Keywords: Endometriosis; IVF/ICSI; Hashimoto Thyroiditis; Fertility outcomes; Pregnancy rates.

Abstract

Objective: To evaluate the prevalence and impact of impaired thyroid-stimulating hormone (TSH) levels on the reproductive outcomes of in vitro fertilization patients diagnosed with endometriosis and compared to controls without endometriosis.

Design: Retrospective cohort study on prospectively collected data

Setting: Tertiary care University Hospital

Participants: Infertile women with histopathological diagnosis of endometriosis.

Methods: for 12 months (January 2018 to January 2019), women were deemed suitable and subsequently divided according to serum thyroid-stimulating hormone levels above or below 2.5 mIU/L and compared to patients without endometriosis. Needed sample size was at least 41 patients for each cohort of women. Co-primary outcomes were the live birth rate (LBR), clinical pregnancy rate (CPR) and pregnancy loss rate (PLR).

Results: 226 women (45 with endometriosis and 181 controls without endometriosis) were included. Diagnoses of Hashimoto thyroiditis were significantly more frequent in women with rather than without endometriosis (14/45 (31.1%) vs 27/181 (14.9%); p=0.012). Similarly, in women with endometriosis, Hashimoto diagnosis rates were higher with TSH ≥2.5 mIU/L compared to TSH <2.5 mIU/L (9/15 (60%) vs 5/30 (16.6%); p=0.001), so were the Hashimoto diagnosis rates in control group (women without endometriosis) with TSH ≥2.5 mIU/L compared to TSH <2.5 mIU/L (17/48 (35.4%) vs 10/133 (7.5%), respectively; p=0.001). Effect size analysis confirmed an increased risk of Hashimoto thyroiditis in women with endometriosis and TSH ≥2.5 mIU/L compared to women with endometriosis and TSH <2.5 mIU/L ((risk ratio (RR) 3.60 (95% CI 1.46 to 8.86)) and in women with endometriosis and TSH ≥2.5 mIU/L compared to non-endometriotic euthyroid patients (RR 7.98 (95% CI 3.86 to 16.48)). Dysmenorrhea risk was higher in endometriotic euthyroid women compared to euthyroid patients with no endometriosis (RR 1.87 (95% CI 1.21 to 2.87)). The risk was still increased in euthyroid women with endometriosis relative to dysthyroid women with no endometriosis (RR 1.97 (95% CI 1.11 to 3.50)). There were no significant differences between the four groups for CPR, LBR, PLR and retrieved oocytes, immature oocytes, degenerated and unfertilized oocytes, cultured blastocysts, embryos and transferred embryos.

Limitations: Retrospective design, limited sample size and use of different ovarian stimulation protocol.

Conclusions: Thyroid autoimmunity seems more common in women with endometriosis and thyroid-stimulating hormone over 2.5 mIU/L. However, there was no significant impact on in vitro fertilization and reproductive outcomes related to the coexistence of endometriosis, Hashimoto disease and higher thyroid-stimulating hormone levels. Due to limitations of the study, additional evidence is required to validate the abovementioned findings.

Introduction

Endometriosis is a persistent inflammatory disorder characterized by the occurrence of tissue that resembles endometrium outside the uterus [1]. According to estimates, it affects more than 10-20% of reproductive-age women and causes lesions in the peritoneum and ovaries, leading to dysmenorrhea, persistent pelvic discomfort, dyspareunia, and infertility [2]. Oestrogen has a significant part in the pathophysiology of endometriosis, making it an oestrogen-dependent condition [3].

Oestrogen also generates systemic and localized inflammation and allows the transplantation of endometrial tissue into the peritoneum, which affects proliferation and immortalization of ectopic endometrial cells [4].

Using self-reported data from mail-in surveys, the Endometriosis Association conducted a large cross-sectional survey in the USA and found that women with endometriosis had substantially greater incidences of autoimmune disorders, including hypothyroidism (9.6% against 1.5% in the overall population) [5].

It has been reported that thyroid autoimmunity and endometriosis could be related, and that among the inflammatory variables, autoimmunity may act a significant part in the development of endometriosis [6, 7]. The prevalence of positive anti-thyroid antibodies has also been associated with endometriosis [8].

Therefore, immunological or endocrine system problems, or both, may be linked to infertility and reproductive impairment since menstrual abnormalities, infertility, and higher pregnancy morbidity are common in women with thyroid disease. A higher incidence of pregnancy losses has also been linked to isolated thyroid autoimmunity, especially in the first trimester. [9, 10].

Endometriosis has been repeatedly linked to several immunological alterations, including complement deposits, autoantibodies to endometrial antigens, a decline in natural killer lymphocytes, and cytotoxic consequences on autologous endometrium [11].

In women with thyroid autoimmunity, endometriosis was more prevalent —25% versus 14% and 44% vs 9% respectively—than in controls, according to studies by Abalovich et al. [12] and Gerhard et al. [13].

Although current evidence seems to stand for a pivotal contribution for autoimmunity in the pathogenesis of endometriosis and a higher incidence of thyroid pathology in women dealing with such disease, data still appear contradictory and conflicting, depicting the necessity for additional evidence to draw firm conclusions [6].

Moreover, even if a relationship between thyroid autoimmunity and endometriosis could be ascertained, it is still debated whether the coexistence of endometriosis and thyroid pathology has a synergistic effect in harming patient's reproductive health by lowering fertility outcomes.

Therefore, the aim of this report was to estimate the prevalence and impact of impaired thyroid-stimulating hormone (TSH) levels and thyroid autoimmunity on the reproductive outcomes of women diagnosed with endometriosis, in comparison with women without endometriosis.

Materials and Methods

Study design

The current investigation was conceived as a retrospective cohort study using data that were acquired prospectively on infertile women who underwent their first cycle of assisted reproductive techniques (ART) at the University Medical Centre of Ljubljana, Department of Human Reproduction, from January 2018 to January 2019. The study procedure complied with the Committee on Publication Ethics' (COPE) standards and the Helsinki Declaration. The protocol's design, data collection, analysis, and interpretation procedures, as well as its first draft and any future updates, all adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement's reporting requirements for observational studies [14].

Inclusion and exclusion criteria

Women in their reproductive years (between the ages of 22 and 41) who had experienced early pregnancy loss, primary or secondary infertility, or both, and who were undergoing diagnostic laparoscopy and ART were included. As defined by the World Health Organization, a woman was deemed infertile if, following a year of consistent, unprotected sexual activity, she was unable to conceive naturally. Based on the Bologna criteria for diminished ovarian reserve, women had to have an adequate ovarian reserve, defined as serum Anti-Mullerian Hormone (AMH) levels of 0.8 ng/ml or higher. [15].

Three or more prior in vitro fertilization (IVF) cycles, hydrosalpinx, untreated endometrial polyps, adhesions or other intrauterine or cervical anomaly, untreated Mullerian anomaly, untreated abnormal PRL or TSH, history of malignancy within five years since enrolment. non-endometriosis laparoscopic surgery, reproductive hormonal treatment within 6 months from surgery were considered as exclusion criteria.

Women were also asked to report the presence of dysmenorrhea, defined as pelvic discomfort or cramps during the menstrual flow (menstrual dysmenorrhea).

Patients were subsequently divided in two groups according to the intraoperative presence or absence of superficial, deep, or ovarian endometriosis. The diagnosis of endometriosis was therefore laparoscopically staged (according to the revised American Society for Reproductive Medicine (rASRM) criteria) and confirmed by histopathological analysis after excision. Only women with histopathological confirmation were included in the study.

According to the Endocrine Society Clinical Practice Guideline, prior to becoming pregnant, a TSH level of less than 2.5 mIU/L is advised [16]. Women receiving levothyroxine treatment had a basal TSH value greater than or equal to 2.5 mIU/L. Consequently, we divided the two groups into two smaller groups: those with TSH levels greater than or equal to 2.5 mIU/L (whether they were taking levothyroxine or not) and those with TSH values lower than 2.5 mIU/L who were on levothyroxine were classified as thyroid dysfunction patients. Patients with TSH levels ≥2.5 mIU/L at enrollment who started levothyroxine and subsequently had modified TSH levels were also classified as thyroid dysfunction patients. Therefore, thyroid-healthy women were defined as those who did not take levothyroxine and had TSH levels less than 2.5 mIU/L.

TSH levels were measured using Roche Elecsys TSH agent for the Roche Cobas e601 module automated analyzer using electrochemiluminescence for immunoassay analysis for the Roche Cobas 6000 analyzer (Roche Molecular Diagnostics Slovensko, s.r.o., Bratislava, Slovakia).

Diagnosis of Hashimoto thyroiditis was made throughout evaluation of thyroid hypofunction, with thyroid autoantibodies (thyroid peroxidase antibodies (TPO-Ab) and thyroglobulin antibodies (Tg-Ab) and thyroid ultrasound. TPO-Ab levels equal or above 35 IU/ml and/or Tg-Ab levels equal or above 40 IU/ml were used as diagnostic thresholds. Ultrasonographic diagnostic features were decreased echogenicity, heterogeneity hypervascularity, small intraglandular cysts, and presence of hypoechoic micronodules with echogenic rim [17].

ART protocol

Ovarian stimulation was carried out by means of short or long gonadotropin-releasing hormone (GnRH) agonist or antagonist protocol. The protocol choice was tailored to patient’s characteristics to maximize the fertility outcomes.

Patients in the short, standardized protocol, from the second day of their menstrual cycle, were daily stimulated using 225 IU of recombinant follicle-stimulating hormone (rFSH) (Puregon; Schering-Plough, Kenilworth, New Jersey, USA). When the dominant follicle measured 13mm in diameter, 0.25mg of the GnRH antagonist cetrorelix acetate (Cetrotide; Merck Serono, Darmstadt, Germany) was injected.

Women in the long protocol were treated with buserelin acetate daily in doses of 0.6 mg (Suprefact; Hoechst AG, Frankfurt/Main, Germany) subcutaneously beginning on day 22 of the menstrual cycle and continuing for 14 days. Controlled ovarian stimulation with rFSH (Puregon; Schering-Plough, Kenilworth, New Jersey, USA) was initiated when the pituitary desensitization requirements were satisfied (estradiol concentration less than 0.07 nmol/L, endometrial thickness less than 4 mm, lack of follicles more than 5 mm).
A minimum number of three leading follicles of minimum 17 mm in diameter to start the final oocyte maturation process were needed. Depending on the overall number of follicles present on the trigger day, oocyte maturation was stimulated. If there were fewer than 20 follicles that were at least 11 mm in diameter, 6500 IU of recombinant hCG (rHCG) (Pregnyl, Organon, Jersey City, New Jersey, USA or Ovitrelle, Merck Serono, Darmstadt, Germany) was used. We administered 0.6 mg of buserelin acetate in a single bolus when there were equal to or more than 20 follicles that were less than 11 mm in diameter.
Transvaginal ultrasound-piloted oocyte retrieval was accomplished 34–36 hours following triggering.
A single bolus of 1500 IU hCG was administered subcutaneously for luteal phase support 60 minutes following the oocyte pick-up to patients with a regular or moderate-high ovarian response (less than 20 oocytes) and a decreased hazard of causing ovarian hyperstimulation syndrome (OHSS). Beginning 24 hours following ovarian puncture and continuing during 12 weeks of gestation, the patients were administered 2 mg of oral estradiol (Estrofem, Novo Nordisk, Bagsvaerd, Denmark) and 200 mg of micronized vaginal progesterone (Utrogestan, Cyndea Pharma SL, Olvega, Spain) every 8 hours. When there was a high risk of OHSS or an abnormal ovarian responsiveness (over 20 oocytes), all good-grade embryos were frozen on days 5 or 6. Luteal assistance was not administered in these situations.
Oocytes were fertilized using intracytoplasmic sperm injection (ICSI) or traditional IVF. In case of severe male factor infertility, ICSI was employed.
On days 3 or 5 following retrieval, no more than two embryos were transferred in accordance with Slovenian national standards for a single embryo transfer. Patients who previously had blastocyst growth breakdown or who only had one or two developed embryos received transfers of day three cleavage stage embryos. Oocytes and embryo quality were graded according to the standardized criteria of Gardner and Schoolcraft [18].

Study variables

The co-primary outcomes of this study were the live-birth rate (LBR), clinical pregnancy rate (CPR), and pregnancy loss rate (PLR), which were defined as follows: LBR was the birth of a live fetus after 24 weeks of gestational age; CPR was ultrasound visualization of one or more intrauterine gestational sacs and fetal heartbeat and PLR was the spontaneous termination of pregnancy before 12 weeks of gestation. Co-secondary outcomes were the following IVF outcomes: oocytes retrieved (defined as number of retrieved oocytes after one procedure), immature oocytes (defined as those having a germinal vesicle or germinal vesicle break down without a polar body (MI stage)), degenerated oocytes (defined as an empty zona pellucida (EZP) or damaged oocyte (fragments of oocytes) inside the zona pellucida) and unfertilized oocytes (defined as the number of non-fertilized eggs after IVF/ICSI), number of cultured blastocysts (defined as the number of formed blastocysts after five days of culture), embryos (defined as the number of embryos that could be possibly transferred to the patient) and transferred embryos (defined as the number of embryos that were transferred to the patient during the embryo transfer procedure).

Data collection

Two databases were used for retrieving study data. The Computerized Database of Reproductive Surgery (CDRS) at the Department of Human Reproduction, containing patients' hospital charts, was used to obtain anonymized information on the clinical and demographic characteristics of women, as well as the type and indication of surgery for endometriosis. Patient's age (median and 95% confidence interval (CI)), body-mass index (BMI) (median and 95% CI), TSH, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) levels (median and 95% CI), Hashimoto disease and dysmenorrhea rate (absolute number and percentage) menstrual cycle length (median and 95% CI) were all extracted from the CDRS (n=898).
The National Register of Assisted Reproductive Technology) (NRART) was used to extract IVF and reproductive outcomes (median number of retrieved, immature, degenerated and unfertilized oocytes (median and 95% CI), number of cultured blastocysts, embryos and transferred embryos (median and 95% CI), LBR, CPR and PLR (absolute number and percentage)). The Republic of Slovenia's Ministry of Health first used NRART in 1983, and since 1999, data have also been routinely provided to the IVF Monitoring (EIM) Consortium of the European Society of Human Reproduction and Embryology (ESHRE).

Only women with complete reporting of all the study variables were included in the analysis; those with missing data from at least one of the two study databases were therefore excluded.

Sample size calculation

The comparison of the co-primary outcome CPR between groups of women with and without endometriosis undergoing IVF/ICSI was used to calculate sample size. The alpha error level was fixed at 0.05, the power was set at 80%, and the group ratio was set at 1 to 4. Pregnancy rates were observed to be 28% in women with concomitant endometriosis and 33% in the group without endometriosis, as previously published [19]. As a result, there should be a minimum of 40 participants in each arm of the study.

Statistical analysis

STATA 14.1 (StataCorp L.L.C., College Station, Texas, USA) was employed for all the analysis. The data were displayed as an absolute number (percentage) or, for continuous variables, as the median with 95% CI, after evaluation of data distribution with the Shapiro-Wilk test. Furthermore, we used Mann-Whitney U or Kruskal-Wallis tests, where appropriate, for comparisons involving continuous variables; for categorical variables, we used Chi-square or Fisher's test for multiple comparisons. For continuous variables, the effect size was expressed as mean difference (MD) with 95% CI and for categorical variables, as risk ratio (RR) with 95% CI. Logistic regression analysis reporting adjusted odds ratio (aOR) for age, BMI and years of infertility was used to evaluate factors (e.g. ovarian stimulation protocol) that might interfere with primary and secondary outcomes. Statistical significance was set at p-value (p) ≤ 0.05.

Results

Among the 898 women undergoing IVF/ICSI in our institution during the study period, 515 matched the inclusion criteria, of those 286 were excluded due to missing TSH information. Therefore, 229 women were initially included. Subsequently, 3 women were removed from the analysis due to missing outcome data. Overall, 226 women were included in the final analysis. Of those, 45 (19.9%) were diagnosed with endometriosis and 181 (80.1%) without. TSH levels were ≥ 2.5 mIU/L in 63 women (27.9%), of which 15 were included in the endometriosis group and 48 in the non-endometriosis group (Figure 1).

Among the 63 patients with thyroid dysfunction, 32 (51%) were treated with levothyroxine. Of those, 7 out of 15 (48%) were diagnosed with endometriosis while 25 out of 48 (52%) were non-endometriotic.

Considering all patients, a multiple blastocyst transfer was carried out in 32 out of 226 (14.2%) women, while the other patients were subjected to a single transfer (85.8%).

There was no significant dissimilarity on baseline features of the two main groups (Table 1). However, there were significantly more cases of Hashimoto Thyroiditis in the endometriosis group relative to the non-endometriosis group (14/45 (31.1%) vs 27/181 (14.9%); p=0.012). When divided by fertilization techniques (IVF or ICSI), there were no differences regarding oocyte and embryo characteristics between endometriotic and non-endometriotic patients (Table 2)

When women were stratified according to the occurrence or lack of endometriosis and serum TSH levels over or under 2.5 mIU/L, there were comparable rates of ICSI or IVF among subgroups (Table 3). In addition, similar serum FSH, LH and PRL levels were found between groups (Table 2). In women with endometriosis, Hashimoto diagnosis rates were more frequent with TSH ≥ 2.5 mIU/L relative to TSH under 2.5 mIU/L (9/15 (60%) vs 5/30 (16.6%); p=0.001). Similarly, the Hashimoto diagnosis rates in the non-endometriosis group were higher with TSH ≥ 2.5 mIU/L relative to TSH under 2.5 mIU/L (17/48 (35.4%) vs 10/133 (7.5%); p=0.001)

Comparing fertility outcomes after IVF did not show a significant distinction concerning retrieved quantity of retrieved oocytes, immature or degenerated oocyte, blastocysts or embryo developed or transferred embryos (Table 3)

Similarly, no significant differences concerning reproductive outcomes (CPR, LBR and PLR) among women with or without endometriosis, and with or without TSH levels over and under 2.5 mIU/L, were found (Table 3).

Table 4 shows the size of effect related to the study's variables among the subgroups reported as RR. There was an increased risk of Hashimoto thyroiditis in women with endometriosis and TSH ≥2.5 mIU/L compared to women with endometriosis and TSH <2.5 mIU/L (RR 3.60 (95% CI 1.46 to 8.86)). The risk continued to increase, reaching almost an 8-folded value, when there was a coexistence between endometriosis and TSH ≥2.5 mIU/L compared to non-endometriotic and euthyroid patients (RR 7.98 (95% CI 3.86 to 16.48)). Accordingly, the risk of dysmenorrhea in euthyroid women was increased when they were diagnosed with endometriosis compared to euthyroid

patients with no endometriosis (RR 1.87 (95% CI 1.21 to 2.87)). The risk was still increased in women with endometriosis and TSH <2.5 mIU/L relative to women with no endometriosis but with TSH ≥2.5 mIU/L (RR 1.97 (95% CI 1.11 to 3.50)) (Figure 2). Conversely, effect size analysis, reported as MD, for FSH, LH, PRL oocytes retrieved, immature, degenerated or unfertilized oocytes, blastocysts developed, obtained and transferred embryos did not report significant differences among subgroup comparisons (Table 4).
The short protocol as reference in logistic regression analysis was used to estimate the influence of the stimulation protocol on IVF and pregnancy outcomes (Table 5). The use of a long protocol was associated with increased number of developed blastocysts in women with no endometriosis and TSH ≥2.5 mIU/L (aOR 2.83 (95% CI 1.02-4.31)). No other significant findings were noted. Regression analysis was not performed in endometriotic patients with TSH ≥2.5 mIU/L since all women in such subgroup underwent short protocol.

Discussion

This retrospective study showed that although higher rates of Hashimoto thyroiditis could be found in women with endometriosis rather than no endometriosis and in women with higher TSH levels, there might be no significant impact on fertility and reproductive outcomes related to the coexistence of endometriosis and thyroid disease.
For the female reproductive system, an adequate quantity of circulating thyroid hormones is crucial [20]. The thyroid condition itself affects ovarian function: indeed, thyroid function and the hypothalamus-pituitary axis are constantly interacting [20]. Thyroid hormone receptors (TR-1 and TR-2) are expressed on the ovarian surface epithelium, follicle and oocytes [21]. TSH receptors and RNA messenger for TR-1 and TR-2 were also discovered in samples derived from endometrial biopsies and from the ovarian epithelium [6]. While deiodinase types 2 and 3 were produced, granulosa cells did not generate type 1, indicating that the peripheral thyroid hormone thyroxin (T4) may be converted [22]. Triiodothyronine (T3) modulates the effects of LH and FSH; several binding sites for T3 have also been discovered in stromal and granulosa cells as well as oocytes [21, 22].
Our findings showed augmented frequencies of Hashimoto disease in women with endometriosis. Venables et al. reported that thyroid autoimmunity had no impact on pregnancy outcomes in women with altered thyroid hormone profile or in euthyroid patients having IVF [23]. However, even if our study did not find an adjuvant effect of dysthyroid pathology in women with endometriosis, the relation between thyroid immunity and the pathophysiology of endometriosis is still debated.
A new study looked into the possible pathophysiological connection between thyroid issues and endometriosis [6]. Endometriotic patients' eutopic and ectopic endometrium exhibit dysregulated transcripts and proteins that regulate thyroid metabolism, which causes ectopic endometrium to resist T3 action and local T4 buildup [5]. On the one hand, T3 and T4 particularly operate to enhance ectopic endometrial cell proliferation and reactive oxygen species generation; on the other hand, TSH works as a proliferative and pro-oxidative hormone on endometria from both endometriosis patients and controls [6]. The results obtained in vitro were furtherly validated by experiments on mice in which it was discovered that endometriotic implants grew larger as thyroid hormone levels increased [6].
Despite there having been a link to endometriosis-related infertility, the molecular explanation for how thyroid dysfunction contributes to the genesis of endometriosis has not yet been established. Ascertained the inflammatory characteristics of endometriosis, it is claimed that autoimmunity could have a role in the aetiopathology of the inflammatory characteristics of endometriosis. To this end, the use of progestogens (e.g. dienogest) as medical treatment acts on multiple levels: together with a moderate inhibition of gonadotropin secretion which induces a hypoestrogenic-hypergestagenic local endocrine environment that causes ectopic endometrium atrophy, it also acts as an immunosuppressive agent capable of blocking both cytokine secretion and action on several immune system pathways [24, 25]. Similarly, discovering patients who have other autoimmune pathologies (e.g. Hashimoto thyroiditis, multiple sclerosis or Sjögren syndrome) as comorbidities, as well as finding autoantibodies to ovarian and endometrial nucleic acid antigens strengthens the idea that the disease might be included in autoimmune multiple antibody disorders [24]. However, unraveling the tangled pathogenesis of endometriosis remains an enigmatic journey.
According to our findings, in addition to the clear evidence showing that menstrual dysmenorrhea in euthyroid women is more common when they were diagnosed with endometriosis, we found that menstrual dysmenorrhea was still significantly more present in euthyroid women with a diagnosis of endometriosis compared to euthyroid patients without a diagnosis of endometriosis. Therefore, dysmenorrhea could be a symptom suggesting further examination to evaluate the presence or absence of endometriosis. However, a recent study showed that the

association between premenstrual spotting and TSH levels has good predictive chances of detecting endometriosis with a diagnostic accuracy of 51.5%. On the contrary, adding dysmenorrhea to the before-mentioned features of the prediction model decreased its accuracy to 50.4% [26]. Therefore, the co-presence of increased TSH levels and premenstrual spotting may better underline a possible diagnosis of endometriosis than with the addition of menstrual dysmenorrhea [26].

Several limitations should be taken into account for a proper data interpretation. Firstly, due to its retrospective design, the study is subject to its common biases, including selection and population biases, which reduce the overall quality of the evidence. However, we tried to reduce such limitation by collecting data prospectively. Secondly, the study was designed to be single centered, reducing the overall generalizability of the findings. Thirdly, although reaching the minimum sample size, the inclusion of a relatively small number of women reduces the robustness of the results. Such limitation might be related to the exclusion of patients with missing data from the analysis which, on the contrary, is a point of strength for the study. Additionally, even if a limited number of women underwent a long rather than a short ovarian stimulation protocol, the use of different approaches might reduce the generalizability of the findings. However, it is noteworthy that regression analysis did not show any influence of the protocol on study outcomes, except for the number of developed blastocysts in women with no endometriosis and TSH ≥2.5 mIU/L. Conversely, an additional limitation is related to the use of a short protocol in every endometriotic women with high TSH levels, avoiding the possibility of performing a deeper analysis of the protocol choice in such subgroup of patients. Nonetheless, current evidence shows no differences in terms of ART and pregnancy outcomes in women with endometriosis according to the use of a hormone pretreatment or the use of a short protocol [27-29]. Lastly, the number of patients excluded due to missing data regarding TSH levels is an additional limitation of study. It should be acknowledged that the inclusion of a wider number of women might interfere with the actual reporting of results. Therefore, further research is needed to validate such findings due to their inherent limitations.

Moreover, the fact that it is the first retrospective analysis of IVF and reproductive results in patients with both thyroid dysfunction and endometriosis represents an additional strength of this study. The histopathological confirmation of endometriosis in the pathology group is a further strength that increases the validity of the analysis.

Conclusions

According to our findings, there may not be a significant adjuvant effect on fertility and reproductive outcomes related to the coexistence of endometriosis and thyroid disease, even though women with endometriosis have higher rates of Hashimoto thyroiditis and higher TSH levels than women without endometriosis. However, due to the limitations of our study, more investigations with greater numbers of women need to be conducted to validate and expand the current knowledge on the effects of thyroid diseases on patients with endometriosis.

Acknowledgements: None.

Statement of Ethics: Study approval statement: The Republic of Slovenia's Medical Ethics Committee gave the approval for the study (approval ID: 0120-609/2021/7).

Consent to participate statement: Each participant in this study received information about the methods and provided their written informed consent to allow data to be collected and analyzed for research. Given that the study had an observational design, the data obtained were anonymised to remove any information that may be used to formally identify the patient.

Conflict of Interest Statement: The authors declare that they have no conflicts of interest to disclose regarding this publication.

Funding sources: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contribution

S Korosec: Protocol/project development; Data analysis; Manuscript writing

G Riemma: Protocol/project development; Data analysis; Manuscript writing

V Salamun: Data analysis; Manuscript writing

A Franko Rutar: Data analysis; Manuscript editing

AS Laganà: Data management; Manuscript editing

V Chiantera: Data management; Manuscript editing
P De Franciscis: Protocol/project development; Manuscript editing
H Ban Frangez: Protocol/project development; Data analysis; Manuscript writing

Data availability statement: Data and material are available at the corresponding author. The data are not publicly available due to privacy or ethical restrictions. Data will be made available on reasonable request. Further enquiries can be directed to the corresponding author.

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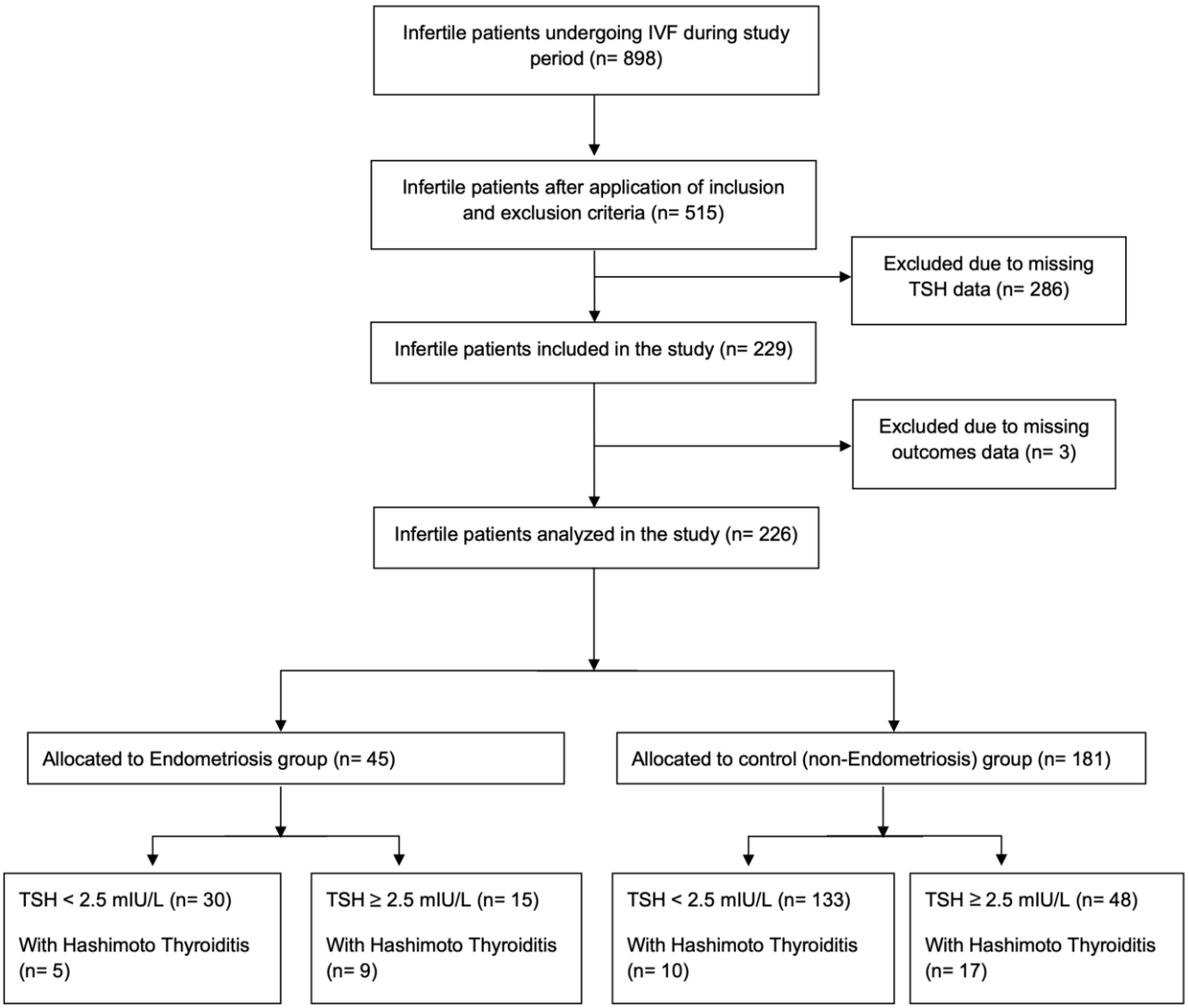
Figure captions

Figure 1. Flowchart of women included in the study according to STROBE guidelines.

Figure 2. Bar graph representation of significant differences among study groups (red dots: risk ratios, grey lines: 95% confidence intervals).



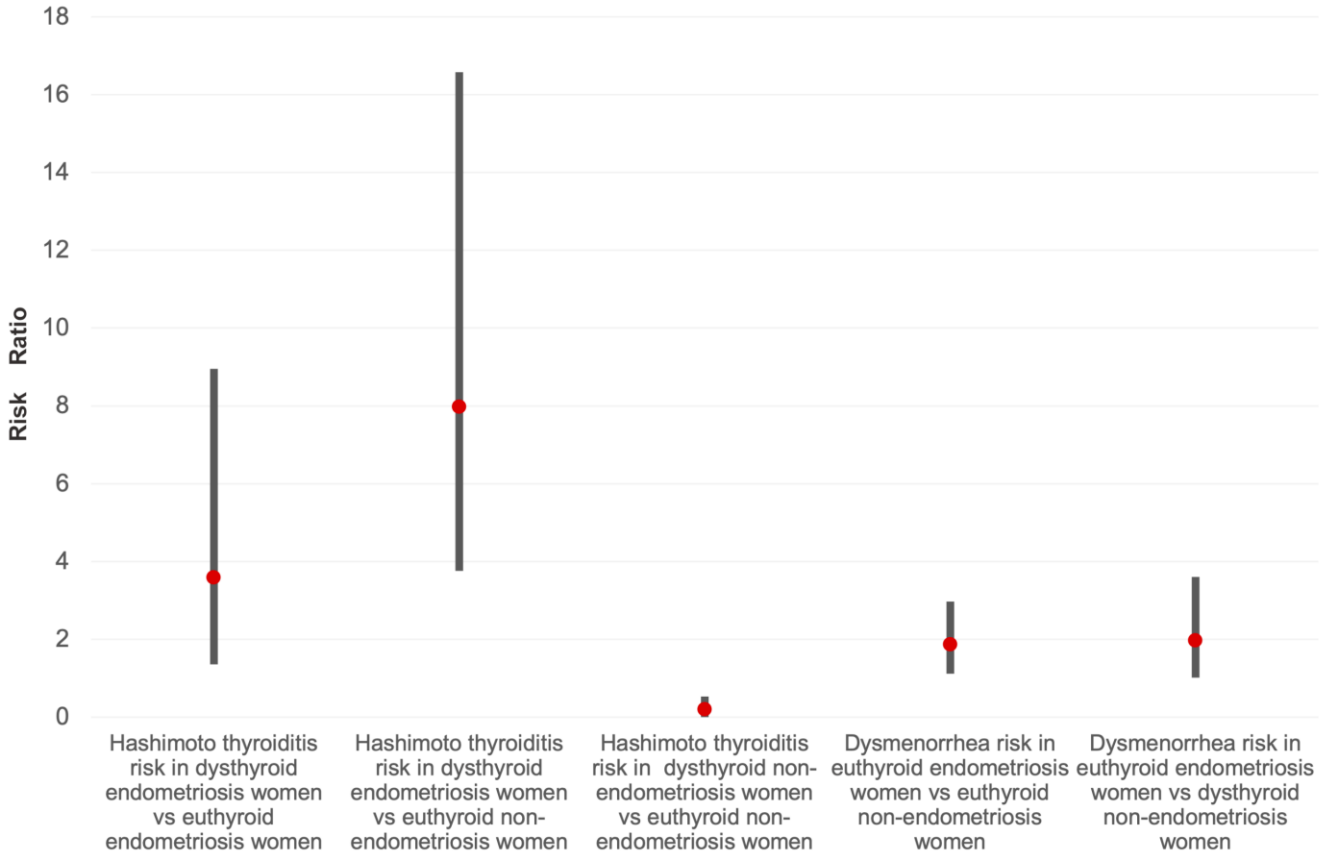


Table 1. Baseline characteristics of infertile patients included in the analysis.

	Endometriosis (n=45)	**Non endometriosis (n=181)**	**P-value**
Age, median (95% CI)	33 (31-35)	33 (32-35)	0.920
BMI, kg/m^2, median (95% CI)	22 (21-23)	23 (22-24)	0.109
TSH under 2.5 mIU/L, n (%)	30/45 (66.7)	133/181 (73.4)	0.335
TSH over 2.5 mIU/L, n (%)	15/45 (33.3)	48/181 (26.6)	0.335
FSH, IU/L, median (95% CI)	7.6 (6.4-8.4)	6.8 (6.4-7.3)	0.610
LH , IU/L, median (95% CI)	4.9 (3.8-5.8)	5.1 (4.6-5.5)	0.968
PRL, ng/mL, median (95% CI)	9.8 (7.5-10.8)	10.1 (9.2-11)	0.749
Hashimoto disease rate, n (%)	14/45 (31.1)	27/181 (14.9)	**0.012**
Dysmenorrhea rate, n (%)	19/45 (42.2)	51/181 (28.1)	0.071
Menstrual cycle length, median days (95% CI)	29 (28-31)	28 (27-30)	0.187
Stimulation protocol			0.267
Long	43 (95.55)	161 (88.95)	
Short	2 (4.45)	20 (11.05)	

CI: confidence interval.

Table 2. Comparison of oocyte and embryo outcomes among IVF and ICSI procedures in women with and without endometriosis.

	Endometriosis		Non Endometriosis		P-value
	IVF	ICSI	IVF	ICSI	
Oocytes retrieved, median (95% CI)	7.0 (5.0-9.5)	7.5 (4.9-10.5)	9 (7.2-10.1)	8.0 (6.2-10.5)	0.499°
Immature oocytes, median (95% CI)	1.0 (0.0-2.0)	1.0 (0-2)	1.5 (1.0-2.0)	1.0 (0-2)	0.215°
Degenerated oocytes, median (95% CI)	0.6 (0-1.0)	0.7 (0-1.0)	0.8 (0.5-1.0)	0.6 (0-1.0)	0.144°
Unfertilized oocytes, median (95% CI)	0.9 (0.5-1.0)	0.8 (0.4-0.9)	1.0 (0.5-1.5)	0.9 (0.5-1.5)	0.638°
Blastocysts developed, median (95% CI)	1.9 (1.2-2.7)	2.0 (1.1-3.1)	1.8 (0.9-2.5)	1.9 (1.3-3.0)	0.955°
Embryos obtained, median (95% CI)	1.7 (0.5-3.0)	2.0 (1.3-2.8)	1.8 (0.9-2.7)	1.8 (1.3-2.5)	0.801°
Embryos transferred, median (95% CI)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.911°

° Kruskal Wallis test; CI: confidence interval.

Table 3. Primary and secondary outcomes of included patients according to serum TSH levels.

	Endometriosis		**Non endometriosis**		**P-value**
	TSH < 2.5 (n=30)	TSH > 2.5 (n=15)	TSH < 2.5 (n=133)	TSH > 2.5 (n=48)	
IVF (%)	14 (46.7)	5 (33.3)	44 (33.1)	10 (20.8)	0.121
ICSI (%)	16 (53.3)	10 (66.7)	99 (66.9)	38 (79.1)	0.121
FSH, IU/L, median (95% CI)	7.7 (6.1-8.4)	7.5 (6.2-8.9)	6.6 (6.4-7.3)	7.1 (6.5-8.4)	0.464°
LH, IU/L, median (95% CI)	4.7 (3.6-6.0)	4.9 (2.7-6.4)	5.3 (4.5-5.7)	4.9 (4.0-5.9)	0.947°
PRL, ng/mL , median (95% CI)	8.0 (6.2-11.0)	9.5 (8.0-14.1)	9.7 (8.4-10.5)	11.8 (10.0-13.4)	0.171°
Hashimoto disease rate (%)	5 (16.6)	9 (60)	10 (7.5)	17 (35.4)	**0.001**^
Dysmenorrhea rate (%)	16 (53.3)	3 (20)	38 (28.6)	13 (27.1)	**0.035**^
Oocytes retrieved, median (95% CI)	7.0 (3.0-11.0)	7.0 (3.0-8.0)	8.0 (6.0-9.2)	9.0 (6.0-12.0)	0.345°
Immature oocytes, median (95% CI)	1 (1.0-2.0)	1.0 (0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.079°
Degenerated oocytes, median (95% CI)	0 (0-1.0)	0 (0-2.0)	0 (0-0.8)	1 (0-1.0)	0.501°
Unfertilized oocytes, median (95% CI)	1.0 (0-1.0)	1.0 (0-2.0)	1.0 (0-1.0)	1.0 (0-1.0)	0.620°
Blastocysts developed, median (95% CI)	1.5 (0-3.0)	0 (0-2.0)	1.0 (0-2.0)	1.0 (0-3.0)	0.555°
Embryos obtained, median (95% CI)	2.0 (1.0-2.0)	2.0 (0-2.0)	1.0 (1.0-2.0)	2.0 (1.0-3.0)	0.120°
Embryos transferred, median (95% CI)	1.0 (1.0-1.0)	.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.329°
Clinical pregnancy rate (%)	11 (36.7)	3 (20)	39 (29.3)	12 (25)	0.609^

Pregnancy loss rate (%)	6 (20)	4 (26.7)	26 (19.5)	8 (16.7)	0.863^
Live birth rate (%)	6 (20)	2 (13.3)	23 (17.3)	8 (16.7)	0.953^

IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; FSH: follicle-stimulating hormone; LH: luteinizing hormone; PRL: prolactin; TSH: thyroid-stimulating hormone; CI: confidence interval.
° Kruskal Wallis test
^ Chi-square test for multiple comparisons

Table 4. Effect sizes for categorical study variables according to comparison between groups (expressed as risk ratios and 95% confidence intervals)

	Endometriosis and TSH <2.5	**Endometriosis and TSH >2.5**	**Non-Endometriosis and TSH <2.5**	**Non-Endometriosis and TSH >2.5**	
Hashimoto disease		NS	NS	NS	**Endometriosis and TSH <2.5**
Dysmenorrhea		NS	NS	NS	
Clinical pregnancy		NS	NS	NS	
Pregnancy loss		NS	NS	NS	
Live birth		NS	NS	NS	
Hashimoto disease	**3.60 (1.46-8.86)**		NS	NS	**Endometriosis and TSH >2.5**
Dysmenorrhea	NS		NS	NS	
Clinical pregnancy	NS		NS	NS	
Pregnancy loss	NS		NS	NS	
Live birth	NS		NS	NS	
Hashimoto disease	NS	**7.98 (3.86-16.48)**		**0.21 (0.10-0.43)**	**Non-Endometriosis and TSH <2.5**
Dysmenorrhea	**1.87 (1.21-2.87**)	NS		NS	
Clinical pregnancy	NS	NS		NS	
Pregnancy loss	NS	NS		NS	
Live birth	NS	NS		NS	
Hashimoto disease	NS	NS	NS		**Non-Endometriosis and TSH >2.5**
Dysmenorrhea	**1.97 (1.11-3.50)**	NS	NS		
Clinical pregnancy	NS	NS	NS		
Pregnancy loss	NS	NS	NS		
Live birth	NS	NS	NS		

TSH: thyroid-stimulating hormone NS: not significant.

Table 5. Logistic regression analysis for study groups according to subtype of stimulation protocol (expressed as adjusted odds ratios and 95% confidence intervals)

	Protocol	Oocytes retrieved	Immature oocytes	Degenerated oocytes	Unfertilized oocytes	Blastocysts developed	Embryos obtained	Embryos transferred	Clinical pregnancy rate	Pregnancy loss rate	Live birth rate
Endometriosis and TSH <2.5	Long	0.85 (0.49-1.13)	1.12 (0.45-1.28)	0.82 (0.06-2.04)	0.77 (0.03-3.19)	0.86 (0.19-2.36)	1.43 (0.60-3.54)	7.78 (0.43-16.91)	2.87 (0.68-6.15)	1.02 (0.99-1.03)	1.01 (0.98-1.02)
	Short	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Non-Endometriosis and TSH <2.5	Long	0.67 (0.19-1.18)	0.52 (0.04-5.28)	5.26 (0.17-16.23)	0.53 (0.03-3.25)	0.56 (0.10-1.44)	0.84 (0.14-1.73)	1.14 (0.19-2.35)	3.22 (0.28-7.29)	1.00 (0.99-1.01)	8.00 (0.68-18.50)
	Short	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Non-Endometriosis and TSH >2.5	Long	1.00 (0.86-1.17)	0.42 (0.18-1.08)	0.70 (0.35-1.25)	0.99 (0.65-1.43)	**2.83 (1.02-4.31)**	0.40 (0.02-1.01)	2.40 (0.82-3.91)	8.08 (0.95-11.73)	2.44 (0.49-11.17)	0.16 (0.01-2.30)
	Short	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference

TSH: thyroid-stimulating hormone