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MORPHOFUNCTIONAL AND IMMUNOHISTOCHEMICAL FEATURES OF THE ENDOMETRIUM IN WOMEN WITH RECURRENT PREGNANCY LOSS AND THYROID HYPOFUNCTION

Akhrorova Sh. O.
Republican Specialized Scientific and Practical Medical Center for Maternal and Child Health, Tashkent, Uzbekistan

Relevance. Recurrent pregnancy loss (RPL) remains one of the most challenging issues in reproductive medicine, affecting up to 5% of women of reproductive age [1]. Despite the multifactorial nature of the condition, growing evidence indicates that endocrine and endometrial factors play a decisive role in recurrent early miscarriages [2]. Among endocrine causes, thyroid dysfunction, particularly hypothyroidism, has been increasingly recognized as a key contributor to reproductive failure [3,4].

Thyroid hormones are essential for the regulation of ovarian steroidogenesis, folliculogenesis, endometrial proliferation, and implantation processes [5]. Even subclinical hypothyroidism can alter gonadotropin secretion, reduce ovarian responsiveness to luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and impair corpus luteum function, leading to luteal phase deficiency and insufficient progesterone secretion [6,7]. These hormonal disruptions result in structural and molecular changes in the endometrium that negatively affect its receptivity during the implantation window.

In recent years, increasing attention has been directed toward the morphofunctional state of the endometrium in women with endocrine disorders. Several studies have shown that thyroid hypofunction is associated with insufficient secretory transformation of the endometrium**,** altered vascularization, and decreased expression of estrogen (ER) and progesterone (PR) receptors [8]. These alterations contribute to a mismatch between embryonic development and the receptive phase of the endometrium, predisposing to early pregnancy loss [9].

Another emerging aspect of endometrial pathology is the role of chronic inflammation and microbiota imbalance. The presence of $CD138^{+}$ plasma cells in the endometrium is now regarded as a reliable marker of chronic endometritis, a condition known to interfere with implantation and early placental development [9,10]. At the same time, disturbances in vaginal microbiota composition — characterized by reduced *Lactobacillus spp.* and increased anaerobic opportunistic flora — have been shown to correlate with inflammatory changes and decreased endometrial receptivity [7]. The interaction between thyroid hypofunction, immune dysregulation, and microbial imbalance remains poorly understood but may represent a key pathogenic link in RPL.

Objective: To evaluate the hormonal, morphofunctional, immunohistochemical, and microbiological features of the endometrium in women with recurrent pregnancy loss (RPL) associated with thyroid hypofunction.

Materials and Methods

A prospective analytical study was conducted from 2022 to 2025 at the Republican Specialized Scientific and Practical Medical Center for Maternal and Child Health. A total of 65 women of reproductive age (18–38 years) were enrolled. The study population included two groups:

- Main group (n = 50): women with a history of recurrent pregnancy loss (RPL) and thyroid hypofunction**;**
- Comparison group (n = 15): women with RPL and normal thyroid function**.**

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Recurrent pregnancy loss was defined as two or more consecutive spontaneous miscarriages before 12 weeks of gestation. All participants provided written informed consent, and the study protocol was approved by the Institutional Ethics Committee in accordance with the Declaration of Helsinki (2013).

Inclusion criteria:

- Women aged 18–38 years with two or more pregnancy losses <12 weeks;
- Regular or irregular menstrual cycles;
- No hormonal therapy within the previous 3 months;
- Absence of uterine structural anomalies or chromosomal abnormalities.

Exclusion criteria:

- Acute pelvic inflammatory disease, endometriosis, or uterine malformations;
- Diabetes mellitus or adrenal disorders;
- Active autoimmune or infectious diseases;
- Recent antibiotic or corticosteroid therapy.

A comprehensive clinical and gynecological evaluation was performed, including detailed reproductive history, anthropometric data (BMI), and menstrual characteristics. Venous blood samples were collected between days 3–5 of the menstrual cycle for the determination of gonadotropins and steroid hormones, and between days 21–23 for progesterone assessment.

The following serum parameters were measured using enzyme-linked immunosorbent assays (ELISA, Roche Diagnostics, Germany):

- Follicle-stimulating hormone (FSH), luteinizing hormone (LH),
- Estradiol (E_2), progesterone (P_4), prolactin,
- Thyroid-stimulating hormone (TSH), free thyroxine (fT_4),
- Anti-thyroid peroxidase antibodies (anti-TPO), total testosterone, DHEA-S, and 17-hydroxyprogesterone (17-OHP).

Reference ranges were adjusted according to laboratory standards. Thyroid hypofunction was diagnosed when TSH > 4.0 μIU/mL and/or fT_4 below 9 pmol/L.

Ultrasound and Doppler Studies

All participants underwent transvaginal ultrasound (GE Voluson E10, USA) to assess uterine size, ovarian morphology, and endometrial thickness (ET) on cycle day 21. Color Doppler imaging of the thyroid gland was also performed to determine thyroid volume and vascular resistance indices (RI, Vmax).

Endometrial tissue was obtained by pipelle biopsy during the mid-luteal phase (days 20–24). Samples were fixed in 10% neutral formalin, embedded in paraffin, and sectioned at 4 μm. Sections were stained with hematoxylin and eosin (H&E) for histological assessment, evaluating glandular-stromal ratios, secretory transformation, vascular patterns, and the presence of inflammatory infiltrates. The diagnosis of chronic endometritis was based on the presence of plasma cells, stromal edema, and vascular sclerosis**.**

Immunohistochemical (IHC) staining was performed using monoclonal antibodies against estrogen receptor (ER)**,** progesterone receptor (PR)**,** and syndecan-1 (CD138) (Dako, Denmark). Antigen retrieval was carried out in citrate buffer (pH 6.0, 95°C, 20 min). Visualization employed the streptavidin–biotin–peroxidase complex method with diaminobenzidine (DAB) chromogen and hematoxylin counterstain.

Expression was evaluated semi-quantitatively using the H-score system: $H = \Sigma Pi\ (i + 1)$, where *i* represents the staining intensity (1–3) and *Pi* the percentage of positive cells. Scores were calculated separately for glandular and stromal components**.**

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The presence of $CD138^+$ plasma cells in stromal tissue was considered diagnostic of chronic endometritis.

The vaginal microbiota composition was studied using a real-time PCR assay "Femoflor-16" (DNA-Technology, Russia). The assay quantified DNA levels of *Lactobacillus spp.*, *Gardnerella vaginalis*, *Atopobium vaginae*, *Ureaplasma spp.*, *Mycoplasma hominis*, and other microorganisms. The Microbial Imbalance Index (IMI) was calculated as the ratio of conditionally pathogenic to normal flora.

Data were processed using Statistica 13.0 (StatSoft, USA) and SPSS 25.0 (IBM, USA). Quantitative parameters were expressed as mean ± standard deviation (M ± SD). Group differences were analyzed using the Student's *t*-test or Mann–Whitney *U* test, depending on data distribution. Correlations between hormonal, morphological, and immunohistochemical variables were assessed using Pearson's or Spearman's correlation coefficients (r). A value of $p < 0.05$ was considered statistically significant.

Results

Clinical and Hormonal Findings. The mean age of women in the main group was 27.1 ± 3.9 years, and in the comparison group 26.8 ± 4.1 years ($p > 0.05$). Body mass index (BMI) ranged from 19.2 to 31.8 kg/m², with overweight or mild obesity in 26% of women with thyroid hypofunction. Menstrual irregularities were significantly more frequent in the main group (56%) than in controls (18%, $p < 0.05$). Oligomenorrhea occurred in 36%, hypomenorrhea in 12%, and secondary amenorrhea in 8% of cases, reflecting luteal phase insufficiency.

Hormonal evaluation revealed elevated TSH and reduced free T_4, estradiol, and progesterone levels in the main group compared with controls, while gonadotropin levels remained within normal ranges.

Table 1. Hormonal characteristics of women with recurrent pregnancy loss depending on thyroid function

Parameter	Main group (RPL + thyroid hypofunction, (n=50)	Comparison group (RPL, n=15)	p
FSH (mIU/mL)	6.9 ± 1.8	6.4 ± 1.6	>0.05
LH (mIU/mL)	5.8 ± 2.0	5.5 ± 1.7	>0.05
Estradiol (pg/mL)	126 ± 32	168 ± 38	>0.05
Progesterone (ng/mL)	8.1 ± 2.3	12.5 ± 3.1	<0.01
Prolactin (mIU/mL)	420 ± 95	360 ± 80	>0.05
TSH (μIU/mL)	5.9 ± 1.4	2.2 ± 0.8	<0.001
Free T_4 (pmol/L)	8.6 ± 1.9	13.2 ± 2.1	<0.001

Elevated TSH correlated inversely with both estradiol ($r = –0.64$, $p < 0.01$) and progesterone ($r = –0.59$, $p < 0.01$), confirming the suppressive influence of thyroid hypofunction on ovarian steroidogenesis.

Histological examination of endometrial biopsies demonstrated insufficient secretory transformation in 68% of women with thyroid hypofunction, compared to 27% in the control group ($p < 0.01$). In the main group, the glands were mostly straight or slightly coiled, lined with cuboidal epithelium, and demonstrated weak cytoplasmic vacuolization. Stromal edema, focal fibrosis, and perivascular lymphoplasmacytic infiltrates were frequent.

Features consistent with chronic endometritis (plasma cells, stromal fibrosis, vascular sclerosis) were found in 42% of the main group and 13% of controls ($p < 0.05$). Endometrial thickness on ultrasound averaged 8.2 ± 1.4 mm in the main group vs. 10.1 ± 1.2 mm in controls ($p < 0.01$).

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These findings indicate morphofunctional immaturity and chronic inflammatory remodeling of the endometrium in hypothyroid women with RPL.

Immunohistochemical staining revealed significant differences in the expression of estrogen (ER) and progesterone (PR) receptors between groups. Women with thyroid hypofunction showed markedly lower H-score values in both glandular and stromal components (Table 2).

The frequency of $CD138^+$ plasma cells was significantly higher in the main group (44%) than in controls (13%, $p < 0.05$). The number of $CD138^+$ cells correlated positively with the presence of morphological signs of chronic endometritis ($r = 0.68$, $p < 0.001$) and inversely with PR expression ($r = –0.49$, $p < 0.05$).

Table 2. Immunohistochemical parameters of endometrial receptor activity and inflammation

Parameter	Main group (n=50)	Comparison group (n=15)	p
ER (glandular, H-score)	165 ± 28	210 ± 35	<0.01
ER (stromal, H-score)	150 ± 24	190 ± 30	<0.01
PR (glandular, H-score)	170 ± 31	240 ± 29	<0.001
PR (stromal, H-score)	160 ± 26	225 ± 27	<0.001
$CD138^+$ cells, % of samples	44.0%	13.3%	<0.05

Lower ER and PR expression was associated with decreased estradiol and progesterone levels, as well as reduced endometrial thickness ($r = 0.62$, $p < 0.01$). The presence of $CD138^+$ cells was linked to diminished receptor expression, supporting the hypothesis of chronic inflammation-mediated receptor desensitization.

Molecular microbiological analysis (Femoflor-16) revealed significant disturbances in the vaginal flora among women with thyroid hypofunction. The total Lactobacillus load was reduced by 1.7 $\log_{10}$ units compared with the control group ($p < 0.05$). Pathogenic anaerobes such as *Gardnerella vaginalis*, *Atopobium vaginae*, and *Ureaplasma spp.* were detected in 52% of the main group versus 18% of controls. The Microbial Imbalance Index (IMI) was elevated (3.4 ± 1.1 vs. 1.8 ± 0.7; $p < 0.01$).

A strong positive correlation was observed between $CD138^+$ cell counts and IMI values ($r = 0.72$, $p < 0.001$), indicating that microbial dysbiosis contributes to chronic endometrial inflammation and decreased receptivity.

Conclusion

This study provides comprehensive evidence that thyroid hypofunction is a significant pathogenic factor in recurrent pregnancy loss (RPL), influencing the endometrium throughhormonal, structural, and immunological mechanisms. Women with thyroid hypofunction exhibited decreased ovarian steroid production, impaired endometrial maturation, and reduced expression of estrogen and progesterone receptors, accompanied by a high prevalence of chronic endometritis ($CD138^+$). Molecular microbiota analysis revealed vaginal dysbiosis characterized by decreased *Lactobacillus spp.* and an increase in anaerobic flora, correlating with inflammatory endometrial changes.

The combined endocrine and inflammatory dysfunctions lead to impaired endometrial receptivity and early reproductive failure. Routine evaluation of thyroid status, together with targeted endometrial and microbiota assessments, should be included in the diagnostic algorithm for women with RPL. A multidisciplinary approach, integrating thyroid hormone correction, luteal support, and anti-inflammatory or probiotic therapy, may improve implantation success and pregnancy outcomes in this group of patients.

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