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Faculty of Medicine
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Study of thyroid function in healthy pregnant women from Plovdiv region and development of population-specific reference limits of thyroid stimulating hormone for each trimester of pregnancy

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The dissertation is presented on 114 standard typewritten pages illustrated with 14 tables, 11 histograms and 6 figures. The bibliography includes 263 authors, 5 of the publications are in Cyrillic and 258 in Latin.

The experimental studies related to the dissertation were carried out at the Clinical Laborotory, University Hospital "St. George", Plovdiv.

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The materials are available in the Scientific Department at MU-Plovdiv, bul. "Vassil Aprilov" 15A and are published on the site of MU-Plovdiv – www.mu-plovdiv.bg

CONTENT

ABBREVIATIONS

ATA – American thyroid association

BMI – body mass index

BSE – Bulgarian society of endocrinology

CI – confidence interval

CLIA – chemiluminescent immunochemical analysis

CLSI – Clinical and laboratory standards institute

ETA – European thyroid association

fT3 – free triiodothyronine

fT4 – free thyroxine

hCG – human chorionic gonadotropin

IFCC – International federation of clinical chemistry

LT4 – levothyroxine

NSEQA – National system for external quality assessment

SD – standard deviation

T3 – triiodothyronine

T4 – thyroxine

Tg – thyroglobulin

TgAb, anti-Tg – thyroglobulin antibodies

TPOAb, anti-TPO – thyroid peroxidase antibodies

TSH – thyroid stimulating hormone, thyrotropin

tT4 – total thyroxine

WHO – World health organization

INTRODUCTION

A normal pregnancy is a state of physiological adaptation and synchronization between the metabolism of a pregnant woman and that of a rapidly growing fetus. The periods during which thyroid diseases most often occur are associated with a specific hormonal attitude of the body – puberty, pregnancy, childbirth, breastfeeding, and climax. Of all these, pregnancy is the highest risk period for overt or hidden manifestation of various thyroid pathology. Pregnancy deepens thyroid abnormalities, which can also affect the development of the future newborn.

During pregnancy, several metabolic, hemodynamic, and immunological changes occur in the thyroid gland, which determine significant differences in thyroid functional indicators of healthy pregnant women relative to the general population. According to literature data, during pregnancy, the reference range of thyroid stimulating hormone (TSH) is smaller than that in non-pregnant women, with a decrease in both the lower (decreased by about 0.1–0.2 mIU/l) and the upper limit for the indicator (decreased by about 0.5–1.0 mIU/l).

Adequate concentrations of thyroid hormones are important for an uncomplicated pregnancy and optimal fetal growth and development. A growing number of studies show that even mild hormonal dysfunction during this period is associated with an increased risk of a several complications and adverse consequences for the mother and fetus. Thyroid hypofunction on the one hand and gestational thyrotoxicosis on the other hand during pregnancy determine the increased incidence of reproductive disorders, miscarriages, anemia, gestational hypertension including intrauterine growth retardation, premature birth, low birth weight and neonatal respiratory distress.

The main diagnostic indicator in thyroid diseases is the measurement of serum thyroid-stimulating hormone and free thyroxine levels, and the optimal approach for interpreting the results is the development and use of population-based reference limits.

To diagnose thyroid dysfunction during pregnancy, it is necessary to use different criteria than those for the general population, due to physiological processes and features affecting the thyroid status during this period. n determining TSH and free thyroxine (fT4) reference ranges, it is essential to eliminate the influence of all factors that have been shown to alter thyroid function. This is critical for correctly identifying women with thyroid dysfunction who would benefit from treatment. That is why the recommendations of several authoritative endocrine organizations such as the American Thyroid Association

and the European Thyroid Association state that trimester-specific values of the indicators for each individual center should be prepared and applied, considering the characteristics of the local population.

The problem of defining reference values of thyroid indicators during pregnancy to serve as criteria for starting treatment and avoiding unnecessary medication is relevant in modern medical practice. Currently, there are no targeted and systematic studies on the problem in Bulgaria and few laboratories have established their own TSH reference limits for the gestation period. The preparation of population-specific reference limits for TSH is a priority issue for endocrinologist, and studies in this direction are encouraged in the consensus opinions of all European and global thyroid organizations.

AIM

Aim of the research:

To study the functional, morphological, and immunological characteristics of the thyroid gland in healthy pregnant women and to develop population-specific reference intervals of TSH and fT4 for the different stages of pregnancy.

TASKS

1. To analyze the demographic and anamnestic characteristics of healthy pregnant women from the Plovdiv region, covered by specialists in the "Maternal Health" program.

2. To test TSH and fT4 levels in healthy pregnant women with negative thyroid antibody titres in the I, II and III trimesters of pregnancy.

3. To analyze the functional thyroid indices of healthy pregnant women dependence on the morphological characteristics of the thyroid gland as assessed by thyroid volumes and physiological changes in thyroid function at different stages of pregnancy.

4. To develop population-specific reference intervals for TSH and fT4 values for the I, II and III trimesters of pregnancy in healthy pregnant women from the studied population.

MATERIAL AND METHODS

I. Material of the study

1. Study design:

Prospective study in the Bulgarian population of pregnant women from the Plovdiv region for each trimester of pregnancy.

2. Selection of the clinical group of healthy persons

The women screened for participation in the study were recruited from outpatient patients, directed by the Obstetrics and Endocrinologists from the Plovdiv region and by the specialists from the Clinic of Endocrinology and metabolic diseases, University Hospital "St. George", Plovdiv. Three groups of 120 pregnant women from each trimester of pregnancy over 18 years were selected to determine reference limits. All participants were selected using previously developed for the purpose of the study questionnaires, medical history, clinical examination by an endocrinologist, ultrasound evaluation of the thyroid gland and laboratory tests. The selection criteria are in accordance with the generally accepted recommendations of the International Federation of Clinical Chemistry (IFCC) and the Clinical and Laboratory Standards Institute (CLSI). Biological material was collected from all of them, and clinical laboratory analyzes were performed at the Clinical Laboratory, University Hospital "St. George", Plovdiv. After pre-selection, 23 of the pregnant women were excluded because of TSH values found outside the reference interval applied to non-pregnant women and/or positive titers for anti-TPO or anti-Tg.

The protocol examinations and examinations of all study participants were performed upon their inclusion after prior signing of the "Informed Consent for Participation". All of them fulfilled the patient selection criteria.

For the conduct of the study, a positive opinion was received from the Scientific Ethics Committee at MU-Plovdiv (e.g. No P-172/21.01.2020), for compliance of the planned scientific study with the standards and criteria for scientificity and accuracy.

PATIENT SELECTION CRITERIA

Inclusion criteria:

- Confirmed pregnancy 1st trimester.
- Age over 18 years.

- A single pregnancy.
- Negative titers for thyroid antibodies (TPOAb, TgAb).
- Abidance
- Signed informed consent form for participation in the study.

Exclusion criteria:

- Pregnancy occurred after applying assisted reproduction methods.
- Taking medications that affect thyroid function.
- Underwent surgical intervention on the thyroid gland or radioiodine treatment.
- Concomitant diseases or conditions that compromise the correct assessment of functional indicators or interfere with laboratory methods for the study of thyroid hormones.
- BMI > 40 kg/m^2.

3. Location of the study

The clinical examinations were carried out at the Clinic of Endocrinology and metabolic diseases, University Hospital "St. George", Plovdiv, and the laboratories in the Clinical Laboratory, University Hospital "St. George", Plovdiv.

4. Characteristics of the studies carried out

Anamnesis was taken and height, weight, BMI were measured for each of the participants in the study, and a clinical and instrumental endocrinological examination was performed.

To extract the primary information, an electronic database was developed in the Excel program of Microsoft Office 2010, which includes a passport part (initials and age of the patients) and the clinical, objective, and clinical laboratory indicators described below.

5. Biological material

In all examined persons, the biological material was taken according to the standard requirements for patient preparation and venous blood collection for clinical-laboratory tests. The preliminary preparation includes a 12-hour food break, a 24-hour break without alcohol and heavy physical exertion, exclusion of coffee and smoking before the procedure. Blood was taken in the morning, at an interval of 7:00 to 9:00 a.m. Before performing the venipuncture, the patient was physically and mentally rested for 15–20 minutes after taking a sitting position. The procedure was carried out in compliance with the conditions of sterility during operation. The following closed venous blood collection systems were

used. To study all hormonal indicators: tubes for taking venous blood (Sarstedt Monovette Serym Z/2.7 ml). The blood for all hormonal parameters is allowed to stand at room temperature from 30 to 45 minutes. The serum is separated immediately after centrifugation at room temperature at RCF 1000 x g/ 3000 rpm for 10 min. Sera with lipemia, hemolysis, and icterus were not included in the study.

6. Work program

The study involved 120 women from the above groups. The anamnesis, the clinical examination, the ultrasound examination of the thyroid gland and the assessment of the risk factors were carried out by specialist's endocrinologists from the Clinic of Endocrinology and metabolic diseases, University Hospital "St. George", Plovdiv. In the Clinical Laboratory, serum concentrations of all listed indicators were determined. The distribution of the planned studies is presented in table 1.

Table 1. Work programme

Visit / **Research**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
	1st trimester /8–13 g.w./	2nd trimester /14–28 g.w./	3rd trimester / > 28 g.w./	up to 3 months after childbirth
Informed consent	☐			
History	☐	☐	☐	☐
Clinical examination	☐	☐	☐	☐
Ultrasonography of the thyroid gland	☐	☐	☐	
TSH	☐	☐	☐	
fT4	☐	☐	☐	
fT3	☐	☐	☐	
anti-TPO	☐			
anti-Tg	☐			

II. Research methods

1. Clinical methods

1.1. Anamnesis

The medical history taken includes detailed pregnancy data (method of pregnancy, sequence, term of pregnancy, single or multiple, date of term), miscarriages, premature births, family history, abidance, bad habits, surgery on the thyroid gland or radioiodine treatment of medication including those affecting thyroid function, as well as clinical signs of hyper and/or hypothyroidism.

1.2. Physical examination – examination of skin and mucous membranes, measurement of pulse and blood pressure, auscultation of the lung and heart, palpation of the thyroid gland, measurement of body mass index, assessment of ocular symptoms (exophthalmos).

1.3. Instrumental methods – ultrasound examination of the thyroid gland at the Clinic of Endocrinology and metabolic diseases, University Hospital "St. George" EAD, Plovdiv.

2. Clinical-laboratory methods

All clinical and laboratory tests were performed in the Clinical Laboratory, University Hospital "St. George", Plovdiv. For all indicators studied, systematic intra-laboratory quality control is carried out through company control materials. External evaluation of the indicators is carried out through participation in the NSEQA and the international program INSTAND, for which the laboratory has the relevant certificates. For the purpose of the study, the determination of serum levels of indicators for assessing thyroid function – thyroid stimulating hormone, free thyroxine, thyroid peroxidase antibodies, thyroglobulin antibodies are performed on an automatic immunological analyzer Access 2® of the American manufacturer Beckman Coulter Inc. by chemiluminescence immunochemical analysis.

Blood for clinical laboratory tests is provided by a closed system for taking biological material (Sarstedt Monovette Serym Z/2.7 ml, cat. LOT № 0033622). The biological material was taken in the morning (between 7:00 and 9:00) on an empty stomach and after compliance with all requirements and preparation for taking biological material. The blood taken, after the formation of a clot, was centrifuged for 10 minutes at 3000 rpm to separate serum. One part of it was immediately used to determine thyroid stimulating hormone (TSH), free thyroxine (FT4), anti TPO- and anti (Tg) antibodies, free triiodothyronine (fT3).

The rest of the serum was placed in a conical plastic tube with an Eppendorf type latch and stored at ≤ -20°C until the analyses were performed.

2.1. Thyroid-stimulating hormone (TSH)

Reagents: CLIA (chemiluminescent immunoassay) – kit of Beckman Coulter, Inc., Ireland.

Principle of the method: two-site immunoenzymatic ("sandwich") method.

Reference limits: 0.34–5.60 mIU/L.

2.2. Free thyroxine (FT4)

Reagents: CLIA (chemiluminescent immunoassay) – kit of Beckman Coulter, Inc., Ireland.

Principle of the method: two-step enzyme immunoassay.

Reference limits: 7.86–14.41 pmol/L.

2.3. Free triiodothyronine (FT3)

Reagents: CLIA (chemiluminescent immunoassay) – kit of Beckman Coulter, Inc., Ireland.

Principle of the method: competitive immunoenzymatic assay.

Reference limits: 3.8–6.0 pmol/L.

2.4. Thyroid peroxidase antibodies (TPOAb)

Reagents: CLIA (chemiluminescent immunoassay) – kit of Beckman Coulter, Inc., Ireland.

Principle of the method: two-step immunoenzymatic ("sandwich") assay.

Reference limits: 0–9 IU/ml.

2.5. Thyroglobulin antibodies (TgAb)

Reagents: CLIA (chemiluminescent immunoassay) – kit of Beckman Coulter, Inc., Ireland.

Principle of the method: method: two-step immunoenzymatic ("sandwich") assay.

Reference limits: 0–4 IU/ml.

3. Statistical methods

Software products – IBM SPSS 26 and REFVAL Program were used for statistical processing of the obtained data.

1. Setting reference limits for TSH and fT4

For each trimester, the reference areas were determined using the method recommended by the IFCC and the Institute of Clinical and Laboratory Standards (CLSI) for the statistical treatment of the reference values the

studied indicators was evaluated by graphical analysis (histograms), formal statistical tests (Kolmogorov-Smirnoff) and analysis of certain skewness and kurtosis coefficients (kurtosis). A parametric method (mean ± 1.96. SD) was applied to determine the reference limits for Gaussian concentration distribution.

In the case of deviation from the normal distribution, the following approaches were applied: using the above-mentioned parametric method, but after transformation of the data, by means of a decimal logarithm operator.

2. Descriptive analysis

For the purposes of descriptive analysis, the following were calculated: arithmetic mean, standard deviation, standard error of the mean (SEM), and in case of deviation from the normal distribution – median.

The following types of graphics were used for graphic visualization: tables, figures, histograms.

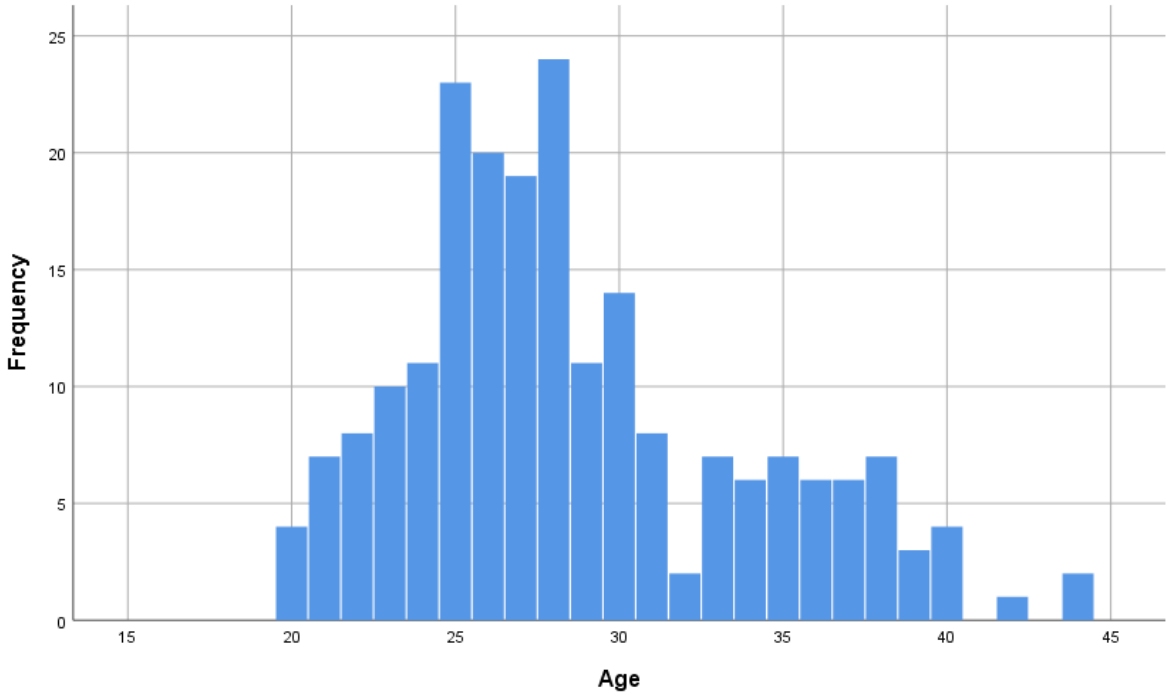
RESULTS

1. Analysis of some demographic and anamnestic factors in healthy pregnant women from the Plovdiv region, covered by specialists in the "Maternal Health" program

1.1. Demographic factors

1.1.1. Age

The study group of pregnant women was 210, 84 of them were observed in two trimesters and 33 in three trimesters of pregnancy. The mean age of the women studied was 28.56 ± 5.11 years. (mean ± SD). The minimum age is 20 years, and the maximum is 44 years. The largest share of pregnant women is in the age range 26–30 years (table 3, histogram 1).



Histogram 1. Age distribution of the observed group of pregnant women

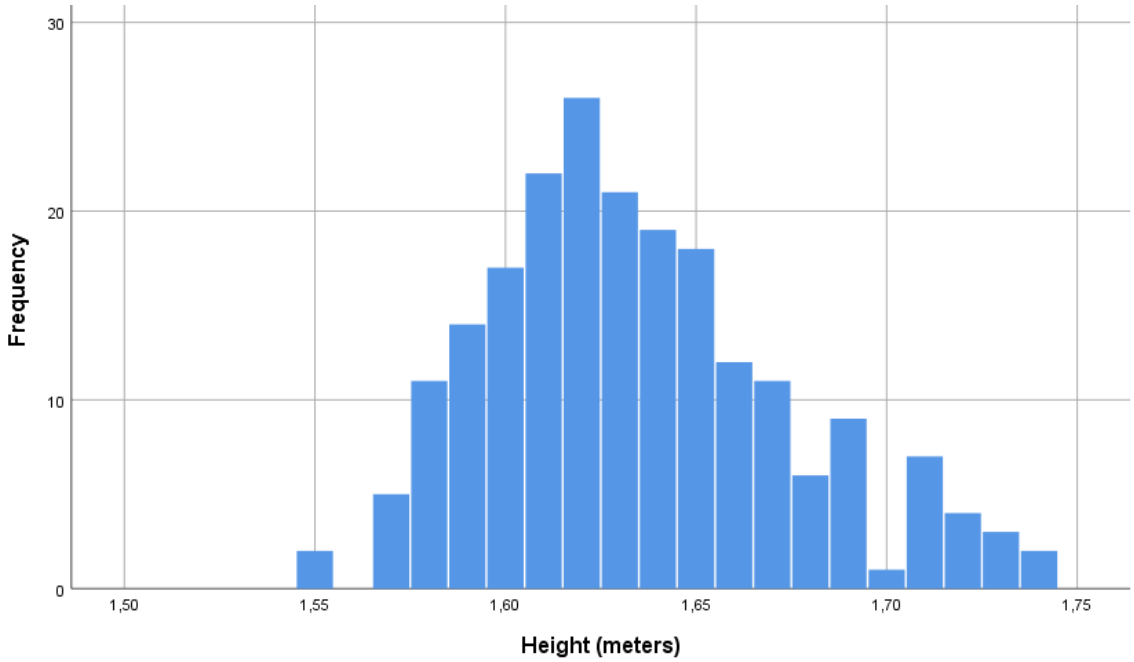
To assess the statistical significance of the relationship between age in years and laboratory performance levels (TSH, fT4 and fT3) for each trimester of pregnancy, a non-parametric analysis was performed, the indicators used to be correlation coefficient and sig. (2-tailed). Data for the first, second and third trimesters did not establish any statistical significance of the relationship difference between the compared magnitudes ($p > 0.05$) (table 1).

Table 1. Evaluation of the statistical significance of the relationship between age in years and laboratory indicators of the thyroid gland during each trimester of pregnancy – nonparametric analysis

Indicator	TSH	fT4	fT3
I st trimester Age in years Correlation Coefficient Sig. (2-tailed)	 -0.040 0.742	 0.218 0.072	 0.195 0.108
n	120	120	120
IInd trimester Age in years Correlation Coefficient Sig. (2-tailed)	 0.079 0.526	 -0.061 0.629	 -0.101 0.433
n	120	120	120
IIIth trimester Age in years Correlation Coefficient Sig. (2-tailed)	 0.227 0.143	 0.241 0.147	 0.052 0.741
n	120	120	120

1.1.2. Height

The study group of pregnant women was 210, 84 of them were observed in two trimesters and 33 in three trimesters of pregnancy. The average for height in meters is 1.64 ± 0.04 m (mean ± SD). The minimum height is 1.55 m and the maximum 1.74 m. The largest is the share of pregnant women in the height interval 1.60–1.65 meters (table 3, histogram 2).



Histogram 2. Height distribution in meters of the observed group of pregnant women

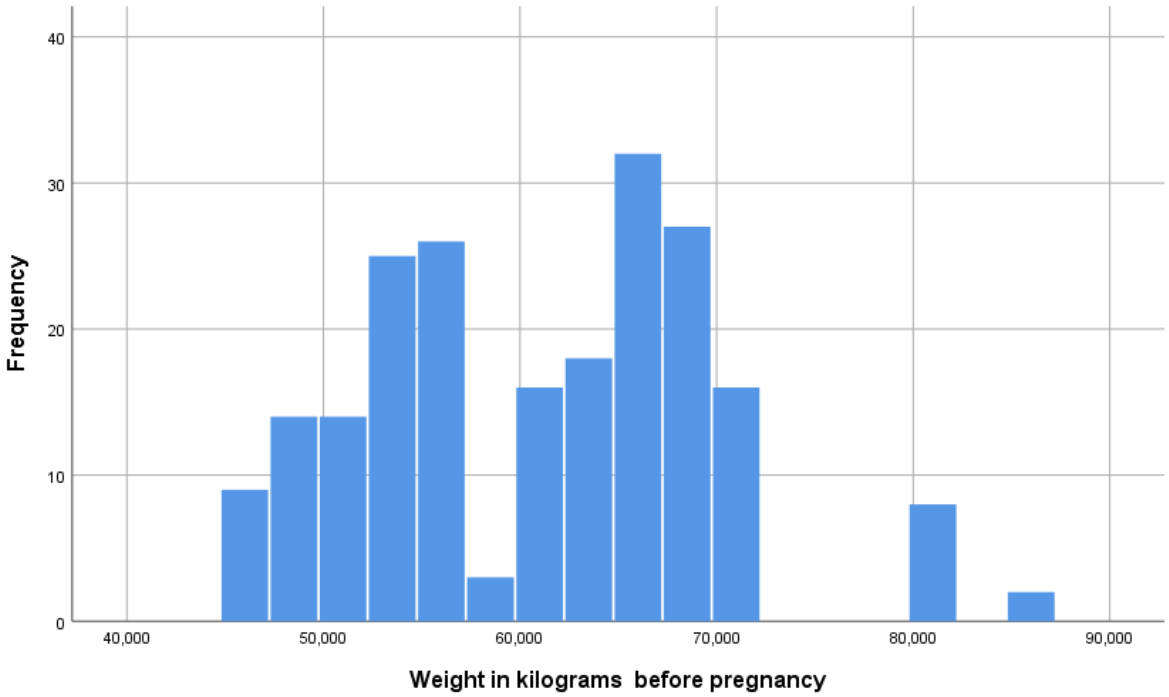
To assess the statistical significance of the relationship between height in meters and laboratory performance levels (TSH, fT4 and fT3) for each trimester of pregnancy, a non-parametric analysis was performed, using the correlation coefficient and sig. (2-tailed). The data for the second trimester established a statistical significance of the relationship between the compared quantities ($p < 0.05$): TSH ($p = 0.027$) and fT3 ($p = 0.013$) (table 2).

Table 2. Evaluation of the statistical significance of the relationship between height in meters and laboratory indicators of the thyroid gland during each trimester of pregnancy – nonparametric analysis

Indicator	TSH	fT4	fT3
I st trimester Growth in metres Correlation Coefficient Sig. (2-tailed)	 0.093 0.447	 -0.043 0.726	 0.002 0.986
n	120	120	120
IInd trimester Growth in metres Correlation Coefficient Sig. (2-tailed)	 0.273 0.027	 -0.080 0.523	 -0.315 0.013
n	120	120	120
IIIth trimester Growth in metres Correlation Coefficient Sig. (2-tailed)	 -0.065 0.679	 -0.030 0.847	 -0.147 0.347
n	120	120	120

1.1.3. Weight before pregnancy

The study group of pregnant women was 210, 84 of them were observed in two trimesters and 33 in three trimesters of pregnancy. The average weight in kilograms of the observed group of pregnant women before pregnancy was 60.62 ± 8.37 kg (mean ± SD). The minimum measured weight is 46.00 kg, and the maximum is 85.00 kg. The largest is the share of pregnant women in the weight interval 60–70 kg (table 3, histogram 3).



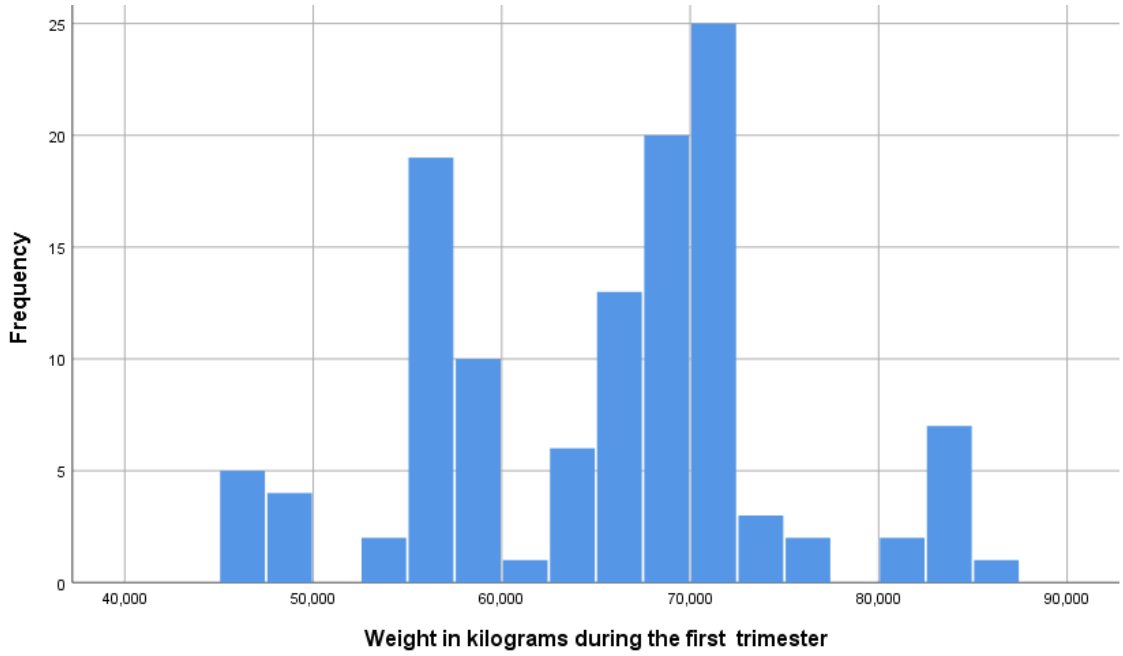
Histogram 3. Distribution of pregnant women of the first, second and third trimesters by weight in kilograms

Table 3. Characteristic of the study group of women

Indicator	min–max	mean ± SD	95% CI
Age (years)	20–44	28.56 ± 5.11	[27.67; 29.46]
Growth (meters)	1.55–1.74	1.64 ± 0.04	[1.63; 1.65]
Weight before pregnancy (kilograms)	46.00–85.00	60.62 ± 8.37	[59.17; 62.10]
Weight during examination in the first trimester (kilograms)	46.00–87.00	65.08 ± 9.12	[63.43; 66.73]
Weight during the examination in the second trimester (kilograms)	49.50–90.00	65.57 ± 9.06	[63.93; 67.21]
Weight during examination in the third trimester (kilograms)	50.00–96.00	74.23 ± 13.50	[70.73; 77.71]

1.1.4. Weight of pregnant women in the first trimester.

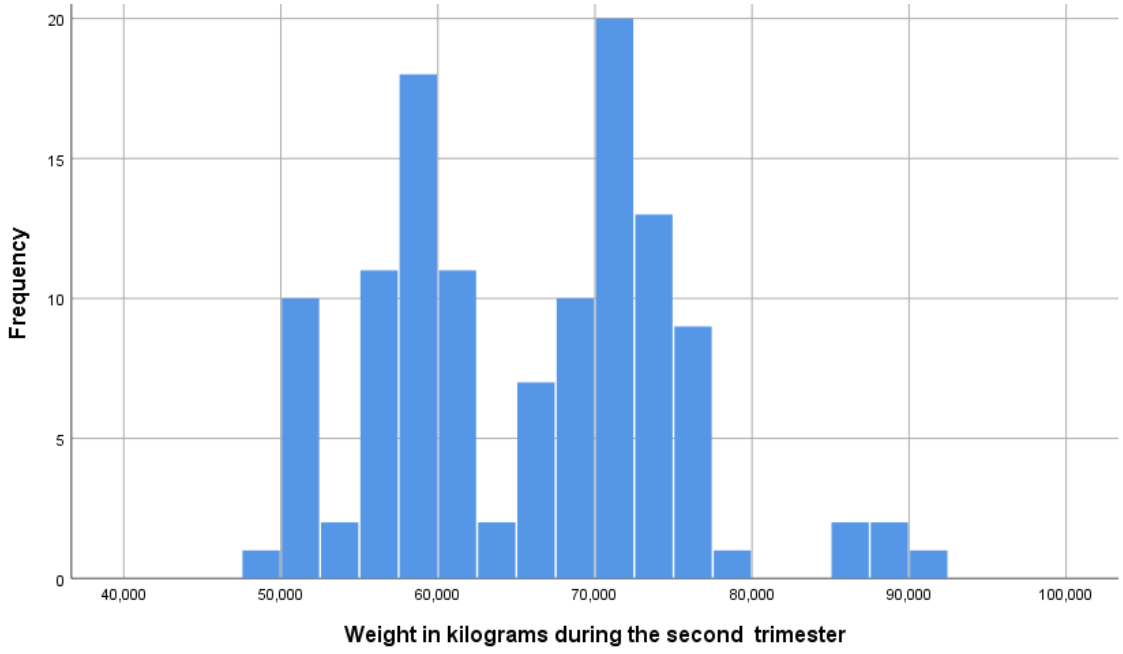
The weight of pregnant women in the first trimester was measured during the clinical examination, with a total number of patients observed being 120. The average value for weight in kilograms is 65.08 ± 9.12 kg (mean ± SD). The difference between the average weight in the first trimester and that before pregnancy 4.46 kg. The minimum measured weight is 46.00 kg, and the maximum is 87.00 kg. The largest is the share of pregnant women in the weight interval 65–75 kg (table 3, histogram 4).



Histogram 4. Weight distribution in kilograms of pregnant women of the first trimester

1.1.5. Weight of pregnant women in the second trimester

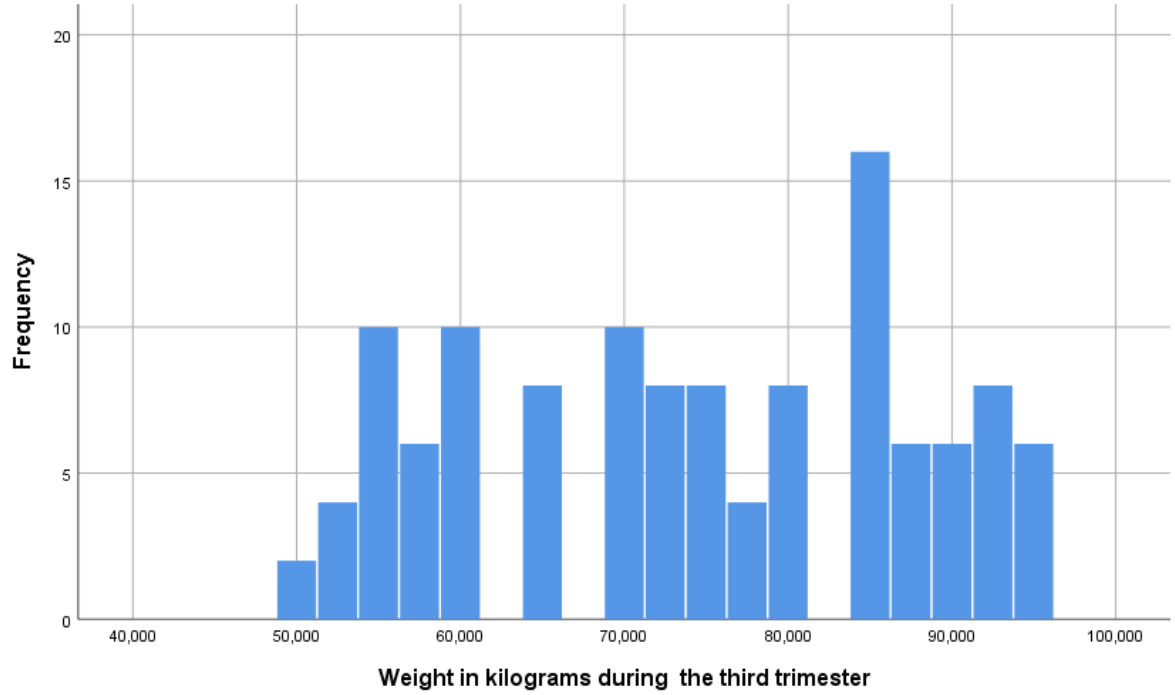
The weight of pregnant women in the second trimester was measured during the clinical examination, with a total number of patients observed being 120. The average value for weight in kilograms is 65.57 ± 9.06 kg (mean ± SD). The difference between the average weight in the second trimester and that before pregnancy is 4.95 kg. The minimum measured weight is 49.500 kg, and the maximum is 90.00 kg. The largest is the share of pregnant women in the weight interval 60–70 kg (table 3, histogram 5).



Histogram 5. Weight distribution in kilograms of pregnant women of the second trimester

1.1.6. Weight of pregnant women in the third trimester

The weight of pregnant women in the third trimester was measured during the clinical examination, with the total number of patients observed being 120 (n = 120). The average value for weight in kilograms is 74.23 ± 13.50 kg (mean ± SD). The difference between the average weight in the third trimester and that before pregnancy is 13.61 kg. The minimum measured weight is 50.00 kg, and the maximum is 96.00 kg. The largest share of pregnant women is in the weight range 70 and 90 kg (table 3, histogram 6).



Histogram 6. Weight distribution in kilograms of pregnant women of the third trimester

To assess the statistical significance of the relationship between weight in kilograms for the respective trimester and laboratory thyroid parameters for the same trimester (TSH, fT4 and fT3) of pregnancy, a non-parametric analysis was performed, the indicators used to be correlation coefficient and sig. (2-tailed). Data for the second trimester ($p < 0.05$): fT4 ($p = 0.038$) as well as the data for the third trimester ($p < 0.05$): fT3 ($p = 0.013$) found a statistical significance of a relationship between the quantities compared (table 4).

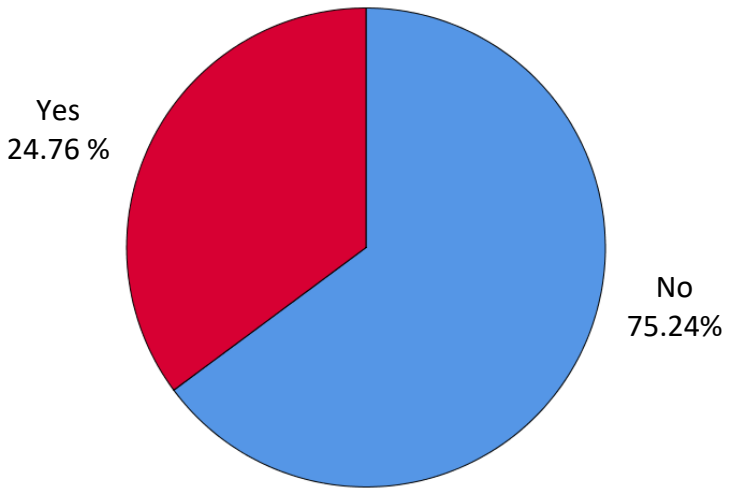
Table 4. Evaluation of the statistical significance of the relationship between weight in kilograms for the respective trimester and laboratory indicators of the thyroid gland for the same trimester of pregnancy – nonparametric analysis

Indicator	TSH	fT4	fT3
Ist trimester Weight in kilograms Correlation Coefficient Sig. (2-tailed)	 0.105 0.393	 -0.051 0.679	 0.021 0.863
n	120	120	120
IInd trimester Weight in kilograms Correlation Coefficient Sig. (2-tailed)	 -0.40 0.68	 -0.274 0.038	 -0.061 0.660
n	120	120	120
IIIth trimester Weight in kilograms Correlation Coefficient Sig. (2-tailed)	 0.046 0.729	 0.179 0.171	 0.411 0.013
n	120	120	120

1.2. Anamnestic factors

1.2.1. Family history of thyroid pathology

The total number of observed women during the three trimesters of pregnancy was 210 (n = 210), 84 of them were observed during two trimesters, and 33 during three trimesters of pregnancy, with 158 (75.24%) reporting no family history of thyroid pathology. In the remaining 52 (24.76%) pregnant women, there was a history of thyroid disease in a close relative (table 5, histogram 7).



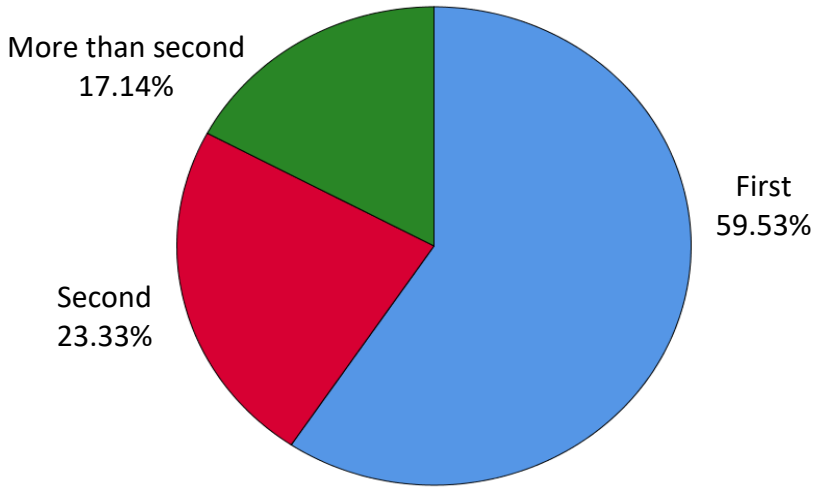
Histogram 7. Percentage distribution of pregnant women with the presence or absence of family history of thyroid pathology

Table 5. Characteristics of anamnestic factors in the study group of women

Characteristic	count	percent
Family history of thyroid pathology		
No	158	75.24
Yes	52	24.76
Sequence of pregnancy		
First	125	59.53
Second	49	23.33
More than second	36	17.14
Infertility		
No	180	85.71
Yes	30	14.29
Miscarriages		
No	184	87.62
Yes	26	12.38
Smoking		
No	163	77.62
Yes	47	22.38

1.2.2. Sequence of pregnancy

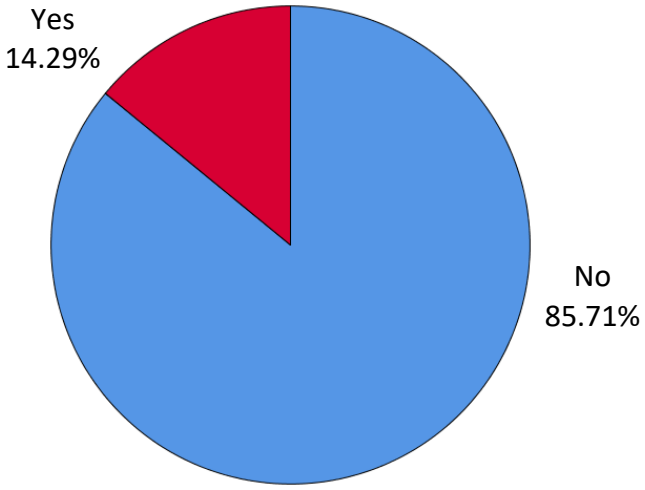
The total number of observed women during the three trimesters of pregnancy was 210 (n = 210), 84 of them were observed during two trimesters, and 33 during three trimesters of pregnancy with 125 (59.53%) of them reporting that this was the first pregnancy, 49 (23.33%) second pregnancy, and in 36 (17.14%) it was more than a second pregnancy. The sequence data of pregnancy are presented in table 5, histogram 8.



Histogram 8. Percentage distribution of pregnant women by sequence of pregnancy

1.2.3. Infertility

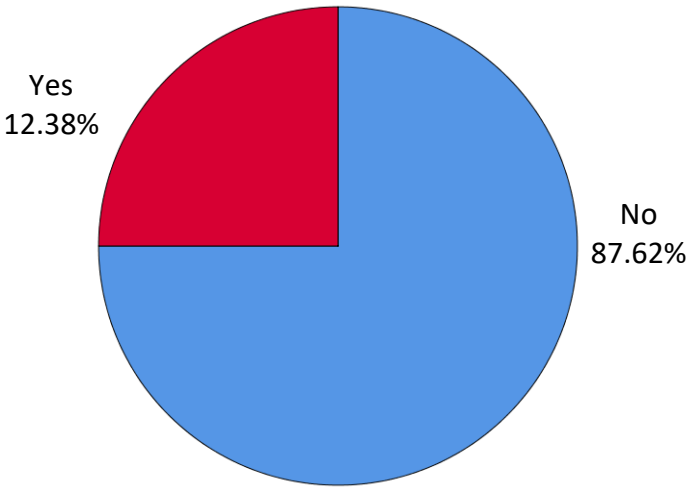
Factor infertility was assessed in 210 (n = 210), 84 of them were observed during two trimesters and 33 during three trimesters of pregnancy. No evidence of infertility was found in 180 (85.71%) of them. The remaining 30 (14.29%) of them reported previous problems getting pregnant (table 5, histogram 9).



Histogram 9. Percentage distribution of pregnant women by infertility factor

1.2.4. Miscarriages

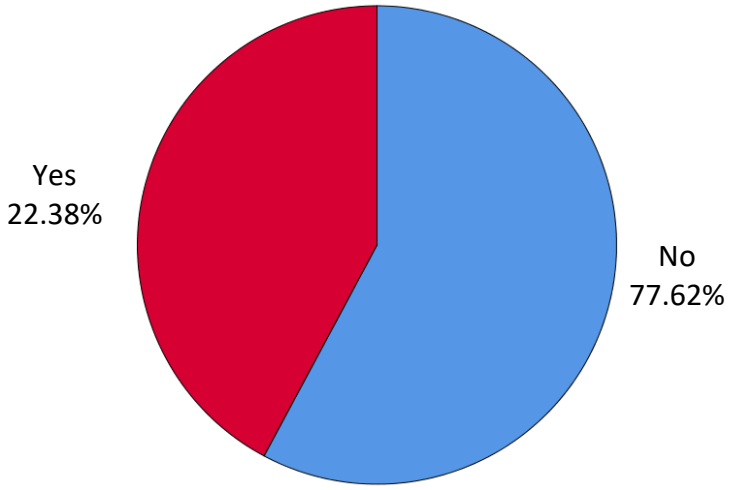
In terms of the miscarriages, a group of 210 (n = 210) pregnant women was assessed, with 84 of them observed during two trimesters and 33 during three trimesters of pregnancy. In 184 (87.62%), no data on previous miscarriages were found. The remaining 26 (12.38%) women reported previous miscarriages (table 5, histogram 10).



Histogram 10. Percentage distribution of pregnant women by the absence or presence of miscarriage

1.2.5. Smoking

In terms of smoking factor, 210 (n = 210) pregnant women were observed, 84 of them observed during two trimesters and 33 during three trimesters of pregnancy. There were 47 active smokers (22.38%), while 163 (77.62%) reported not smoking (table 5, histogram 11).



Histogram 11. Percentage distribution of pregnant women by smoking factor

2. Examination of TSH and fT4 levels in healthy pregnant women with negative titers of thyroid antibodies in the first, second and third trimesters of pregnancy

The first trimester covers the period up to and including the 13th week of gestation. The number of pregnant women studied during this trimester was 120 (n = 120) and were covered between the 5th and 13th weeks of gestation. The share of those who were examined in the 11th week of gestation is the largest. The second trimester covers the period from the 14th to the 28th week of gestation inclusive. The total number of women observed in the second trimester was 120 (n = 120), and they were examined between the 15th and 28th weeks of gestation. The third trimester covers the period after the 28th week of gestation. The studied pregnant women during this trimester were 120 (n = 120) and were covered between the 29th and 37th week of gestation (figure 1).

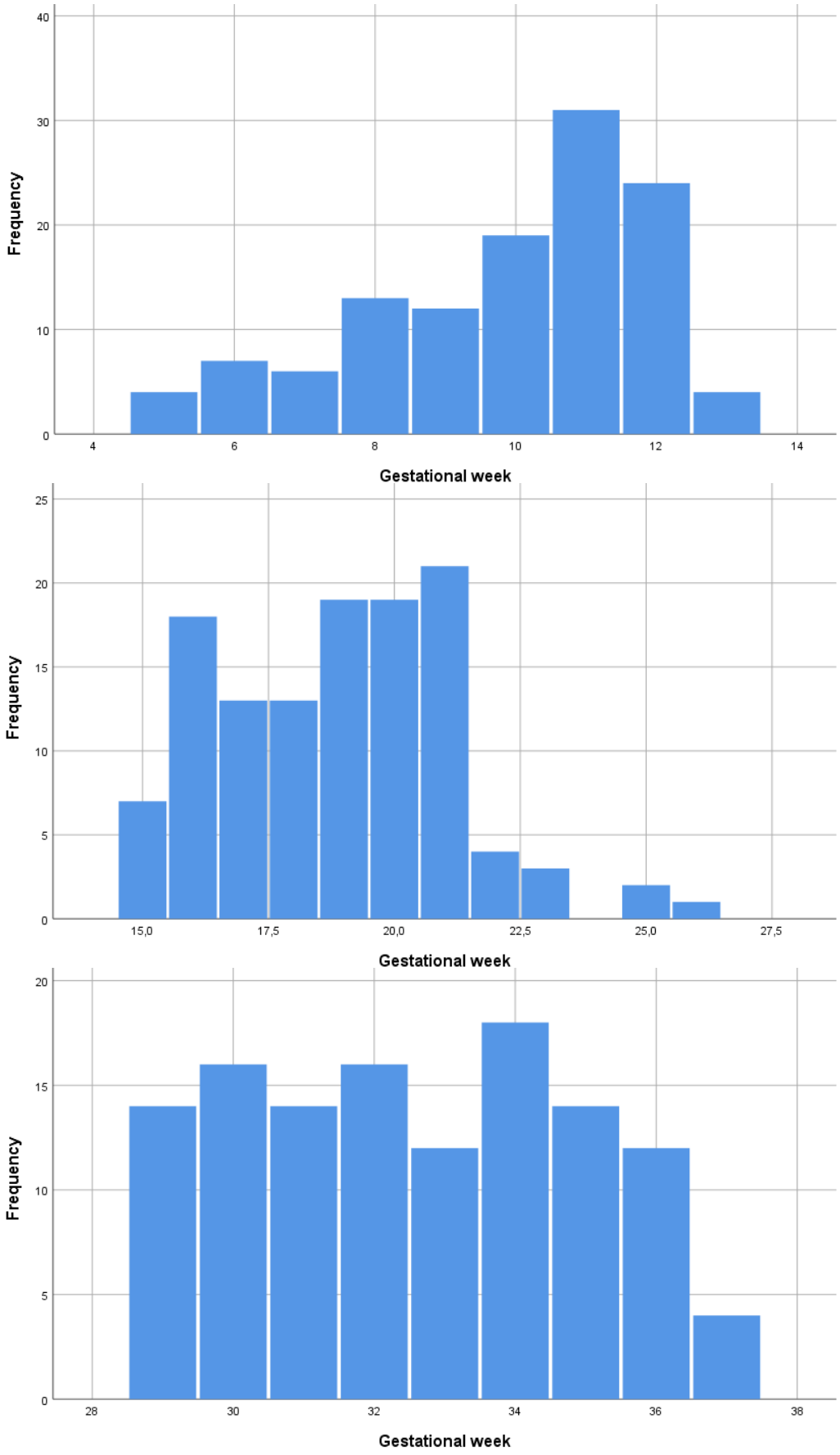


Figure 1. Histograms of the distribution by gestational weeks of pregnant women from the first (n = 120), second (n = 120) and third (n = 120) trimesters

2.1. Determination of anti-TPO and anti-Tg levels in the selected group of pregnant women in the first, second and third trimesters of pregnancy

The following statistical parameters were used to estimate the distribution of anti-TPO and anti-Tg values: mean ± SD, min, max, 95% CI. At anti-TPO for the first trimester, the following parameters were determined 0.43 ± 0.60 IU/ml (mean ± SD), 0.25 IU/ml (min), 3.58 IU/ml (max), [0.29; 0.56] (95% CI). Values below 9 IU/ml are considered negative. At anti-Tg for the first trimester, 0.99 ± 0.52 IU/ml (mean ± SD), 0.90 IU/ml (min), 3.31 IU/ml (max), [0.87; 1.12] (95% CI) were determined. Results below 4 IU/ml are considered negative. The observed females in the first trimester were 120 (table 6).

For the second trimester were determined, anti-TPO was determined – 1.08 ± 1.84 IU/ml (mean ± SD), 0.25 IU/ml (min), 8.30 IU/ml (max), [0.61; 1.56] (95% CI); anti-Tg 1.01 ± 0.37 IU/ml (mean ± SD), 0.90 IU/ml (min), 2.50 IU/ml (max), [0.92; 1.11] (95% CI). The observed females in the second trimester were 120 (table 6).

For the third trimester were determined, anti-TPO was determined – 0.57 ± 1.15 IU/ml (mean ± SD), 0.25 IU/ml (min), 6.97 IU/ml (max), [0.29; 0.87] (95% CI); anti-Tg 1.03 ± 0.45 IU/ml (mean ± SD), 0.90 IU/ml (min), 3.37 IU/ml (max), [0.91; 1.15] (95% CI). The observed females in the third trimester were 120 (table 6).

Table 6. Mean concentrations of anti-TPO and anti-Tg in the study group of pregnant women in the I-st, II-nd, III-th trimester of pregnancy, descriptive statistical parameters for the observed group

Indicator	n	mean ± SD	min	max	95% CI
I st trimester					
anti-TPO	120	0.43 ± 0.60	0.25	3.58	[0.29; 0.56]
anti-Tg	120	0.99 ± 0.52	0.90	3.31	[0.87; 1.12]
II nd trimester					
anti-TPO	120	1.08 ± 1.84	0.25	8.30	[0.61; 1.56]
anti-Tg	120	1.01 ± 0.37	0.90	2.50	[0.92; 1.11]
III th trimester					
anti-TPO	120	0.57 ± 1.15	0.25	6.97	[0.29; 0.87]
anti-Tg	120	1.03 ± 0.45	0.90	3.37	[0.91; 1.15]

2.2. Determination of TSH and fT4 levels in the selected group of pregnant women in the first, second, and third trimesters of pregnancy

The following statistical parameters were used to estimate the distribution of TSH and fT4 values: mean ± SD, min, max, 95% CI, median. The number of women observed in the first, second and third trimesters is 120. At TSH for the first trimester, 1.826 ± 0.889 (mean ± SD), 0.056 (min), 3.668 (max), [1.68; 1.98] (95% CI), 1.723 (median) were determined. Reference interval for TSH according to data of the method used was 0.34–5.6 mIU/l. At fT4 for the first trimester, 9.57 ± 1.74 (mean ± SD), 6.27 (min), 12.76 (max), [9.27; 9.86] (95% CI), 9.86 (median) were determined. The reference interval for fT4 according to data of the method used is 7.86–14.41 pmol/l (table 7).

For the second trimester, TSH 2.591 ± 1.125 (mean ± SD), 0.766 (min), 4.787 (max), [2.40; 2.79] (95% CI), 2.336 (median), fT4 – 7.27 ± 1.95 (mean ± SD), 4.20 (min), 12.64 (max), [6.93; 7.60] (95% CI), 6.90 (median) (Table 7).

For the third trimester, TSH 2.538 ± 1.228 (mean ± SD), 0.895 (min), 5.434 (max), [2.23; 2.85] (95% CI), 2.524 (median) were determined; fT4 – 7.57 ± 2.24 (mean ± SD), 4.13 (min), 11.60 (max), [6.99; 8.14] (95% CI), 7.96 (median) (table 7).

Table 7. Mean TSH and fT4 values in the study group of pregnant women in the I-st, II-nd and III-th trimesters of pregnancy, descriptive statistical parameters for the observed group

Indicator	n	mean ± SD	min	max	95% CI	median
I st trimester						
TSH	120	1.826 ± 0.889	0.056	3.668	[1.68; 1.98]	1.723
fT4	120	9.57 ± 1.74	6.27	12.76	[9.27; 9.86]	9.86
II nd trimester						
TSH	120	2.591 ± 1.125	0.766	4.787	[2.40; 2.79]	2.336
fT4	120	7.27 ± 1.95	4.20	12.64	[6.93; 7.60]	6.90
III th trimester						
TSH	120	2.538 ± 1.228	0.895	5.434	[2.23; 2.85]	2.524
fT4	120	7.57 ± 2.24	4.13	11.60	[6.99; 8.14]	7.96

3. Analysis of functional thyroid indicators of healthy pregnant women dependence on the morphological characteristics of the thyroid gland assessed by thyroid volumes and physiological changes in thyroid function at different stages of pregnancy

3.1. Analysis of thyroid volumes during the first trimester of pregnancy

The observed group of women during this period was n = 120. The thyroid volume during the first trimester of pregnancy averaged 7.19 ± 1.73 ml (mean ± SD), with the minimum measured value being 4.12 ml and the maximum 9.90 ml. The ninety-five percent confidence interval was [6.77; 7.60]. To assess the statistical significance of the relationship between thyroid volumes and the levels of laboratory indicators (TSH, fT4 and fT3) during the first trimester of pregnancy, a non-parametric analysis was performed, using correlation coefficient and sig. (2-tailed). The data for the first trimester established a statistically significant relationship between the compared quantities ($p < 0.05$): TSH ($p = 0.019$), fT4 ($p = 0.040$) and fT3 ($p = 0.028$) (table 8 and table 9).

Table 8. Mean values of thyroid volumes in the study group of healthy pregnant women in the 1st and 2nd and 3rd trimesters of pregnancy, descriptive statistical parameters for the observed group

Indicator	n	mean ± SD	min	max	95% CI
Ist trimester Thyroid volume	120	7.19 ± 1.73	4.12	9.90	[6.77; 7.60]
IInd trimester Thyroid volume	120	7.57 ± 1.95	4.27	11.50	[7.09; 8.05]
IIIth trimester Thyroid volume	120	7.60 ± 1.70	5.09	11.69	[7.16; 8.04]

Table 9. Evaluation of the statistical significance of the relationship between thyroid volumes and laboratory thyroid indicators during each trimester of pregnancy – nonparametric analysis

Indicator	TSH	fT4	fT3
I st trimester Thyroid volume Correlation Coefficient Sig. (2-tailed)	 -0.282 0.019	 0.247 0.040	 0.265 0.028
n	120	120	120
II nd trimester Thyroid volume Correlation Coefficient Sig. (2-tailed)	 -0.024 0.846	 -0.199 0.109	 -0.050 0.698
n	120	120	120
III th trimester Thyroid volume Correlation Coefficient Sig. (2-tailed)	 -0.009 0.945	 -0.112 0.394	 0.103 0.434
n	120	120	120

3.2. Analysis of thyroid volumes in the second trimester of pregnancy

The observed group of women during this period was n = 120. The thyroid volume in the second trimester of pregnancy averaged 7.57 ± 1.95 ml (mean ± SD), with the minimum measured value being 4.27 ml and the maximum 11.50 ml. The ninety-five percent confidence interval was [7.09; 8.05]. To assess the statistical significance of the relationship between thyroid volumes and laboratory parameters (TSH, fT4 and fT3) of the thyroid gland in the second trimester of pregnancy, a non-parametric analysis was performed using the correlation coefficient and sig. (2-tailed). No statistical significance of the relationship ($p > 0.05$) was found in the observed indicators for the second trimester: TSH ($p = 0.846$), fT4 ($p = 0.109$) and fT3 ($p = 0.698$) (table 8 and table 9).

3.3. Analysis of thyroid volumes during the third trimester of pregnancy

The observed group of women during this period was n = 120. The thyroid volume during the third trimester of pregnancy averaged 7.60 ± 1.70 ml (mean ± SD), with the minimum measured value being 5.09 ml and the maximum 11.69 ml. The ninety-five percent confidence interval was [7.16; 8.04]. To assess the statistical significance of the relationship between thyroid volumes and laboratory performance levels (TSH, fT4 and fT3) in the third trimester of pregnancy, a nonparametric analysis was performed, using the correlation coefficient and sig. (2-tailed). No statistical significance of the relationship was

($p > 0.05$) was found in the observed third trimester indicators: TSH ($p = 0.945$), fT4 ($p = 0.394$) and fT3 ($p = 0.434$) (table 8 and table 9).

4. Definition of population-specific reference intervals.

Population-specific reference intervals were determined from the TSH and fT4 values obtained so far for the I-st, II-nd and III-rd trimesters of pregnancy in healthy pregnant women in the study population.

The boundaries of the population-specific reference area have been determined according to the method of statistical treatment of reference values recommended by the International Federation of Clinical Chemistry and the Institute of Clinical and Laboratory Standards.

The type of statistical distribution of the concentration values of the measured indicators for the first, second and third trimesters of pregnancy was investigated. For this purpose, an assessment was made based on histograms, Kolmogorov-Smirnoff test, coefficient of asymmetry (skewness) and kurtosis (kurtosis), P-value. The values of the skewness and kurtosis coefficients and the corresponding P-value values from the formal statistical hypotheses are indicated in table 10. Deviation from the normal distribution was found for the observed parameters for the first and second and third trimesters.

Table 10. Assessment of the type of distribution of the studied indicators in pregnant women of the first (n = 120), second (n = 120) and third trimester (120)

Indicator	N	Asymmetry coefficient (skewness) ±SE	Coefficient of excise (κurtosis) ±SE	One-simple Kolmogorov-Smirnov test	P-value
I st trimester TSH	120	0.119 ± 0.206	-0.901 ± 0.410	0.116	0.000
I st trimester fT4	120	-0.070 ± 0.209	-1.113 ± 0.413	0.145	0.001
II nd trimester TSH	120	0.431 ± 0.211	-0.859 ± 0.419	0.117	0.009
II nd trimester fT4	120	0.601 ± 0.211	0.156 ± 0.419	0.099	0.030
III th trimester TSH	120	0.996 ± 0.304	0.542 ± 0.438	0.174	0.000
III th trimester fT4	120	-0.084 ± 0.304	-1.070 ± 0.599	0.181	0.006

The following descriptive statistical parameters were assigned to the TSH and fT4 indicators studied for the three trimesters of pregnancy: minimum value (min), maximum value (max), arithmetic mean ± standard deviation (mean ± SD), 95% confidence interval (95% CI) and median. The parameters indicated and their values are presented in table 7.

To calculate the reference intervals of TSH and fT4 for each trimester of pregnancy, the parametric method was used, and analysis of the results of the descriptive statistical parameters and the normality of the distribution was carried out in advance. In the case of deviation from the normal distribution, the following approaches were applied: using the above-mentioned parametric method, but after transformation of the data, by means of a decimal logarithm operator.

4.1. Reference intervals for TSH and fT4 in the first trimester of pregnancy.

For the selection of a statistical method in calculating the TSH and fT4 reference intervals during the first trimester of pregnancy, the results of the descriptive statistical parameters and the normality of the distribution were used. For each of the two measured indicators, a reference value was calculated in the corresponding measurement unit and a 90% confidence interval (table 11).

The results of 120 healthy pregnant women obtained to date were used in the preparation of the TSH and fT4 reference ranges for the first trimester of pregnancy. The distribution of concentrations of these indicators is demonstrated in figure 2.

Table 11. Reference limits for TSH and fT4 during the first trimester of pregnancy (parametric estimation two-stage transformation method)

Indicator	Ref. Values mIU/l	90% confidence interval
I st trimester TSH Lower limit Upper limit	 0.318 3.486	 [0.173; 0.475] [3.283; 3.681]
	Ref. Values pmol/l	90% confidence interval
I st trimester fT4 Lower limit Upper limit	 6.51 12.44	 [6.24; 6.80] [12.18; 12.67]

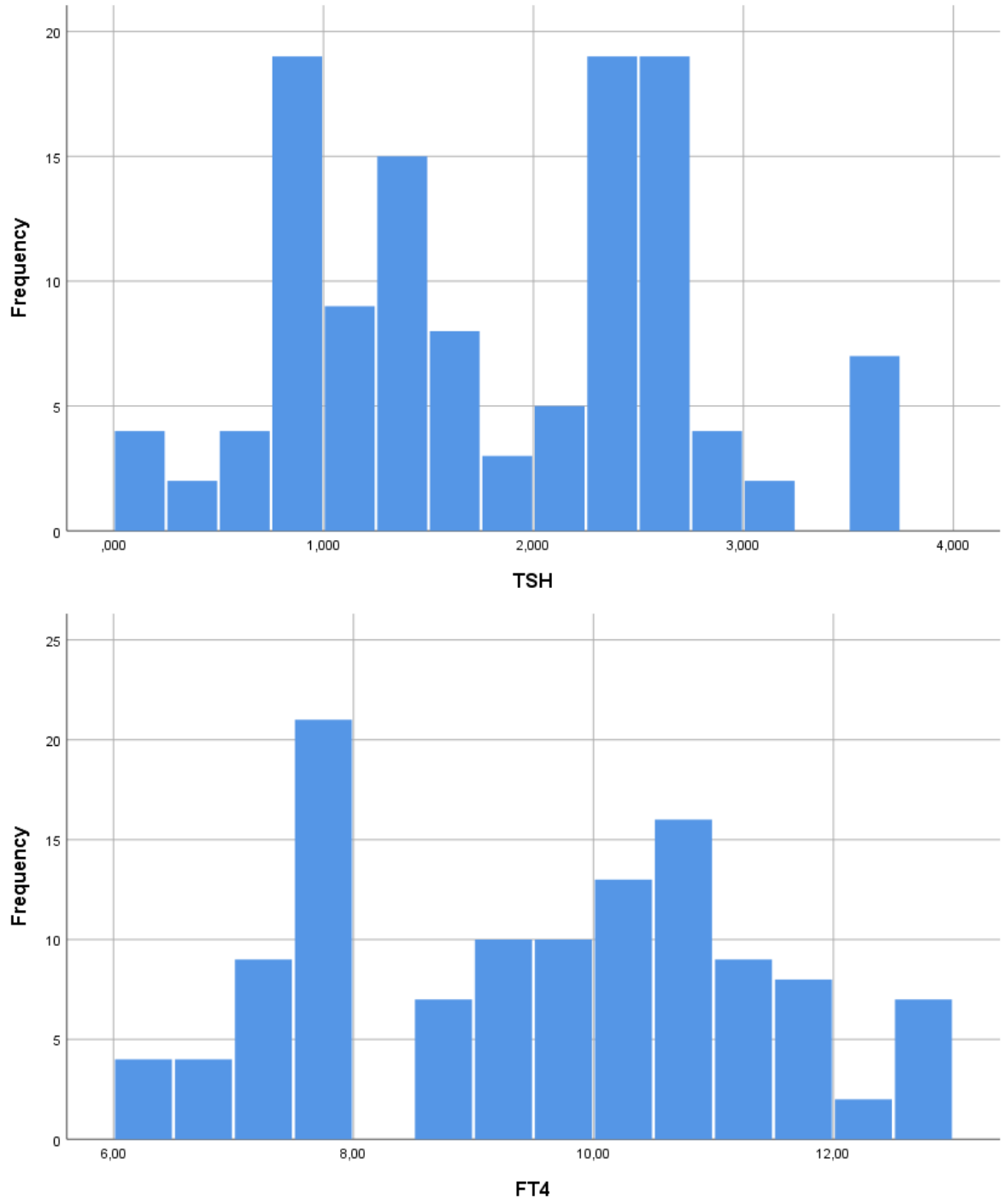


Figure 2. Histograms of the distribution of TSH and fT4 concentrations in pregnant women of the first trimester (n = 120)

4.2. Reference intervals for TSH and fT4 in the second trimester of pregnancy

In preparing the TSH and fT4 reference limits for the second trimester of pregnancy, the results of 120 healthy pregnant women obtained so far were used. The distribution of the concentrations of these indicators is demonstrated in figure 3.

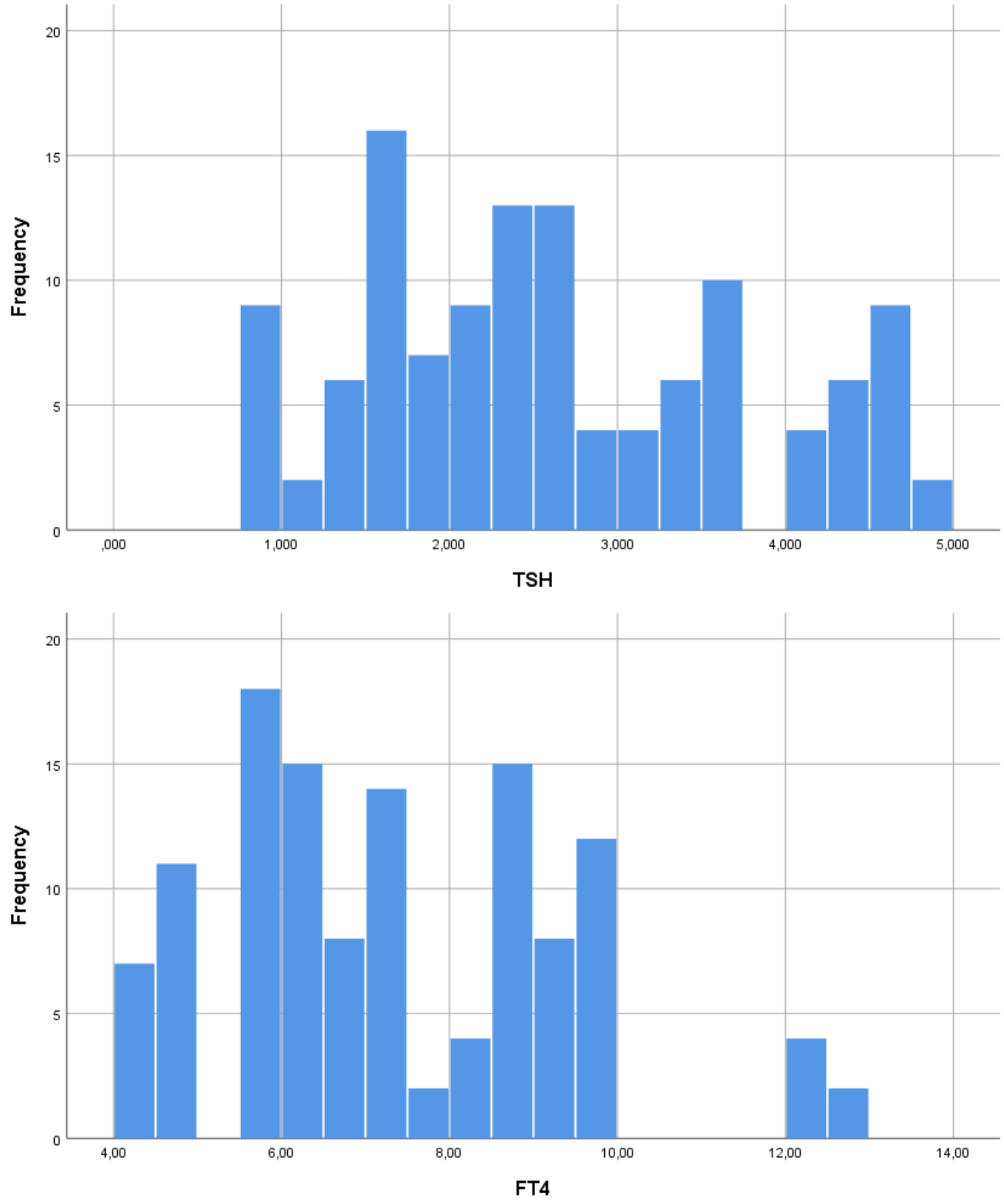


Figure 3. Histograms of the distribution of TSH and fT4 concentrations in pregnant second trimester women (n = 120)

In the calculation of the reference intervals of TSH and fT4 in the second trimester of pregnancy, the parametric method was used, after evaluating the results obtained from the descriptive statistical parameters and analyzing the normality of the distribution. For each of the two measured indicators, a reference value was calculated in the corresponding measurement unit and a 90% confidence interval (table 12).

Table 12. Reference limits for TSH and fT4 during the second trimester of pregnancy (parametric estimation two-stage transformation method)

Indicator	Ref. Values mIU/l	90% confidence interval
II nd trimester TSH		
Lower limit	0.947	[0.839; 1.067]
Upper limit	4.897	[4.583; 5.209]
	Ref. Values pmol/l	90% confidence interval
II nd trimester fT4		
Lower limit	4.26	[4.00; 4.55]
Upper limit	11.45	[10.75; 12.18]

4.3. Reference intervals for TSH and fT4 in the third trimester of pregnancy

In preparing the TSH and fT4 reference limits for the third trimester of pregnancy, the results of 120 healthy pregnant women obtained so far were used. The distribution of the concentrations of these indicators is demonstrated in figure 4.

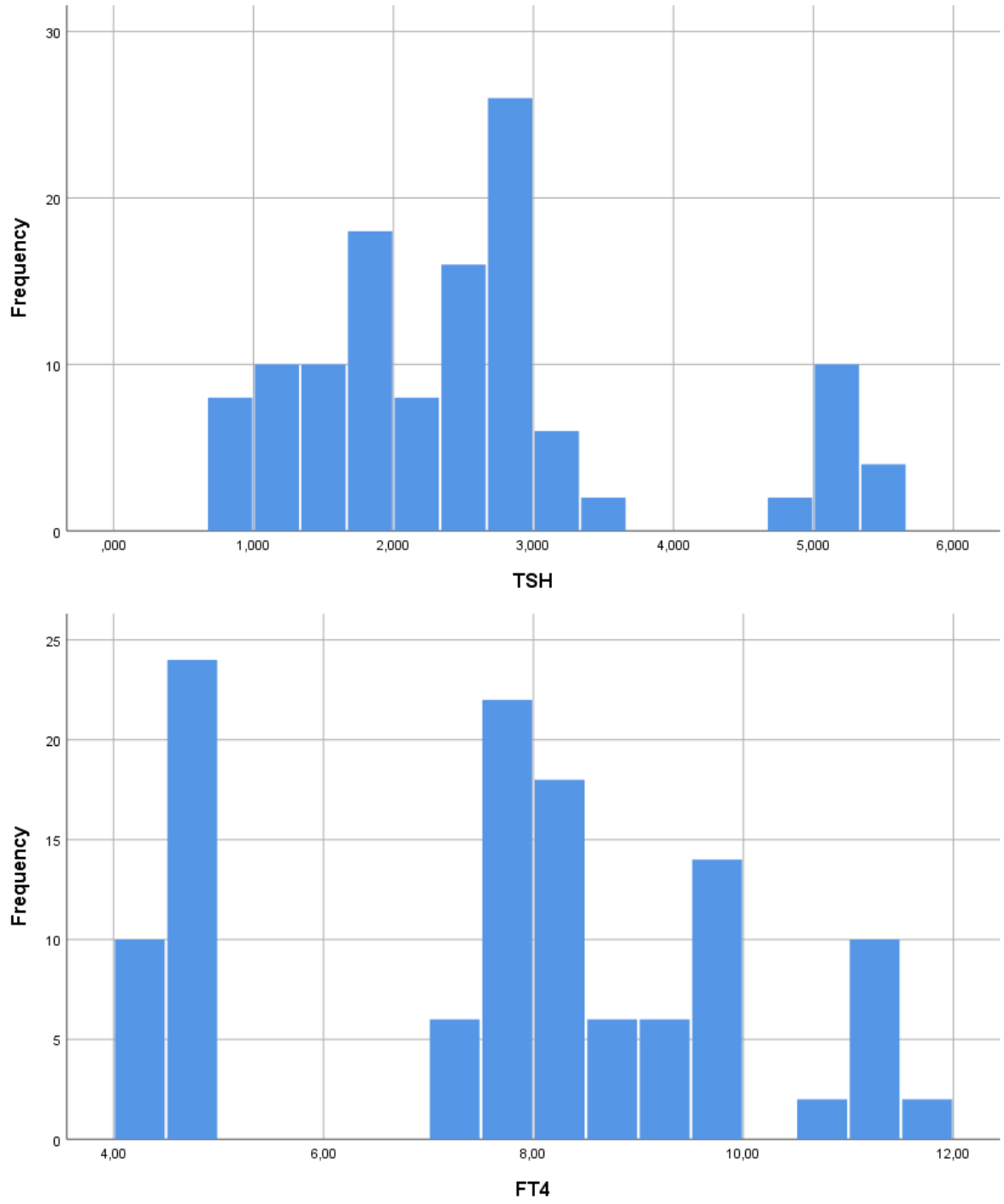


Figure 4. Histograms of the distribution of TSH and fT4 concentrations in pregnant third trimester women (n = 120)

In the calculation of the reference intervals of TSH and fT4 during the third trimester of pregnancy, the parametric method was used, after evaluating the results obtained from the descriptive statistical parameters and analyzing the normality of the distribution. A reference value in the relevant unit and a 90% confidence interval were calculated for each of the two indicators measured (table 13).

Table 13. Reference limits for TSH and fT4 during the third trimester of pregnancy (parametric estimation two-stage transformation method)

Indicator	Ref. Values mIU/l	90% confidence interval
III rd trimester TSH Lower limit Upper limit	 0.888 5.391	 [0.731; 1.073] [4.582; 6.145]
	Ref. Values pmol/l	90% confidence interval
III rd trimester fT4 Lower limit Upper limit	 3.68 11.17	 [3.23; 4.20] [10.75; 11.54]

DISCUSSION

Pregnancy is a stress for the thyroid gland because significant functional changes in the gland occur during this period. Knowledge of physiological thyroid adaptation during this period is essential in evaluating thyroid dysfunction. The normal functioning of the thyroid gland is important for the cardiovascular system, oxygen consumption and basic metabolism, carbohydrate metabolism, stimulation of poplar production, normal growth and development of tissues and organs. Thyroid hormones also have specific physiological effects on the fetus and newborn and are indispensable for its normal development, maturation, and normal brain function. During the fetal period and in infancy, they affect the development of the cerebral and cerebellar cortex and basal ganglion, axon proliferation, synapogenesis, dendritic branching and myelination of nerve fibers, wakefulness state, reactivity, memory and learning ability, as well as the emotional tone of the child. A correlation between children's intelligence and the level of maternal thyroid hormones during pregnancy has been proven.

In the first 10–12 weeks of pregnancy, the fetus is completely dependent on maternal thyroid hormones that cross the placenta. Towards the end of the first 3 months, the fetus begins its own synthesis of thyroid hormones. However, it remains dependent on maternal hormones and the amount of iodine required for the synthesis of thyroxine and triiodothyronine.

Pregnancy affects the thyroid gland and its function. There are a series of changes related to the physiological adaptation of the maternal gland, which occur in the different stages of pregnancy. They provide for the increased but physiological needs of the mother and fetus during this period.

During pregnancy, the thyroid gland increases in size by 10% in iodine-rich countries, and by 20% to 40% in iodine-deficient areas. The daily need for iodine increases, as well as placental deiodinase activity and renal clearance of iodine. In the first trimester, maternal hCG directly stimulates the TSH receptor, increasing the production of thyroid hormones, resulting in a subsequent decrease in serum TSH concentration. The structural features of this dimeric glycopeptide determine the weak TSH-like activity of the hormone. After conception, circulating thyroxine binding globulin and total T4 increase by 7 weeks gestation and reach their peak around approximately 16 weeks gestation. TSH secretion is stimulated until a new equilibrium between the total and free fraction of T4 is established. The production of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), increase by nearly 50%, due to increased estrogens, which in turn stimulate the hepatic production of thyroxine-binding globulin and reduce

the free (bioactive) fraction of the hormone in the circulation. A healthy thyroid gland adapts to these features by altering hormone metabolism, iodine absorption and the regulation of the hypothalamus-pituitary-thyroid axis.

Pregnant women are the vulnerable part of the population. Disorders in the functioning of the thyroid gland are among the most common endocrine diseases during pregnancy. They occur in about 2–4% of the general population manifested as hypo or hyperfunction.

For the purposes of the study, the determination of the serum levels of indicators for the evaluation of thyroid function – thyroid-stimulating hormone, free thyroxine, thyroid peroxidase antibodies, thyroglobulin antibodies were performed on an automatic immunological analyzer Access 2® of the American manufacturer Beckman Coulter Inc. by chemiluminescent immunochemical assay.

The laboratory conducts daily intra-laboratory quality control for all investigated indicators. External evaluation of the quality of the indicators is carried out through participation in NSEQA and international program INSTAND, for which the laboratory has the relevant certificates.

Processing of data from observed demographic and history factors in healthy pregnant women included in this study was carried out. Analysis of the data found that an average age of 28 years. Is comparable to the data from the National Statistical Institute for 2022. Comparable to the literature data are the results of 12.38% found in the study on spontaneous pregnancy loss. In 14.29% of pregnant women observed, there was evidence of difficulty in becoming pregnant. A similar percentage is described by the European Thyroid Association. The established percentage of 24.76 for familiarity with respect to thyroidal pathology is like that described by other authors. The percentage of pregnant women active smokers found in this study was 22.38%. The data are like those for a country published in other studies.

In assessing the statistical significance of the relationship between height in meters and laboratory performance levels (TSH, fT4 and fT3) for each trimester of pregnancy, such significance ($p < 0.05$) was found: TSH ($p = 0.027$) and fT3 ($p = 0.013$) for the second trimester.

The statistical significance of the relationship between weight in kilograms for the respective trimester and laboratory thyroid parameters for the same trimester (fT4 and fT3) for the second trimester ($p < 0.05$): fT4 ($p = 0.038$) as well as for the third trimester ($p < 0.05$): fT3 ($p = 0.013$) was established.

All pregnant women in the monitored group were assigned anti-TPO and anti-Tg levels. The following statistical parameters were used to assess the distribution of anti-TPO and anti-Tg values: mean ± SD, min, max, 95% CI. All women enrolled in the observation had negative thyroid antibody titers compared to the reference intervals we administered. At anti-TPO values below 9 IU/ml were considered negative. For anti-Tg, results below 4 IU/ml were considered negative.

Analysis of the measured TSH concentrations in the observed group of pregnant women for the entire period of pregnancy found that in the first trimester three of them (n = 3) are below the lower reference limit. The values are compared with the reference interval of 0.34–5.6 mIU/l applied by us so far for the entire period of pregnancy. According to some studies, serum TSH below 0.1 mIU/l (in some cases even undetectable) may be present in approximately 5% of women by 11 weeks gestation. Geralyn Lambert et al in their study involving 9,562 women in the first and second trimesters reported an arithmetic mean of the 5th percentile of 0.13 for the first and 0.36 for the second trimester at $p < 0.0001$. The authors found that at least one of the 75 paired samples had a value below 0.01 mIU/l. During pregnancy, women have lower serum TSH concentrations than the pre-pregnancy period and TSH below a lower limit of 0.4 mIU/l for non-pregnant women is observed in 15% of healthy women during the first trimester of pregnancy. Pregnant women with suppressed TSH were up to about 10% in the second trimester and 5% in the third trimester. Although the decrease in TSH in the reference range was observed in all populations, the extent of the decrease varied significantly between different races and ethnic groups.

Pregnant women generally have lower concentrations of fT4 than non-pregnant women. Our first-trimester study at fT4 found an arithmetic mean of 9.57 pmol/l minimum value of 6.27 pmol/l and a maximum of 12.76 pmol/l which are lower than the reference interval of the currently applied method 7.6–14.41 pmol/l. In the second trimester, the distribution was the following arithmetic mean of 7.27 pmol/l, minimum value 4.20 pmol/l, maximum 12.64 pmol/l, and for the third arithmetic mean value of 7.57 pmol/l minimum value 4.13 pmol/l, maximum 11.60 pmol/l. In contrast to the data, we received Yanachkova et al. In their 2019 study, they obtained a first-trimester average of 15.202 pmol/l, a second-trimester average of 13.509 pmol/l, and a third trimester of 14.002 pmol/l. Geralyn Lambert et al found that free T4 values were higher in the first trimester than in the second trimester for all their percentiles of $p < 0.0001$. The differences in the results obtained in these studies convincingly

indicate the need also to set population reference limits for fT4. All of the women seen in the study were also measured with fT3 levels in order to confirm the euthyroid thyroid conditions.

The volume of the thyroid gland is established both palpatory and ultrasound. It is believed that thyroid volume during pregnancy is affected by various factors – iodine replacement, genetic factors, age, TSH, anthropometric parameters, number of births, smoking, etc. According to the World Health Organization (WHO), normal thyroid volume in women should be between 4.4–18 ml. The 2017 Thyroid Ultrasonography Atlas listed an acceptable upper limit below 17.4 ml, and according to M. Boyanov, a norm for the average volume of the thyroid gland in healthy women (15–74 years of age) of 12.4 ± 3.34 mL (9.06–15.74 mL) has been adopted for the Bulgarian population. In the age sub analysis for the 20–29 group, the thyroid volume on average was 11.73 mL (median 11.58 mL), and for the age 30–44 g – 13.6 mL (median 12.93 mL). In our study, thyroid volumes exceeding the accepted norms were not found in any trimester, but a slight increase in all observed parameters was found in the second and third trimesters compared to the first. Similar are the results of Fister et al. and of Özdikici et al., who found an increase in thyroid volume during pregnancy and the difference was significant between the first and third trimesters. Borisova et al. in their 2019 study also found a significant difference in thyroid volume between the first and third trimesters as well as a correlation between thyroid volume and consecutive trimesters. They assume that thyroid volume is probably not affected by TSH given the lower bound levels of the hormone during pregnancy. According to the results of their study, the authors (Borisova et al.) conclude that the thyroid volume in pregnant Bulgarian women in over 97% of cases is normal and directly reflects the established normal average iodine in the urine in them. Its slight increase during pregnancy is an expression of physiological adaptation, in response to a slight iodine deficiency in this period of increased needs. This is also suggested by our study. Fister P. et al. in their study proved that in areas with sufficient iodine, thyroid volume increased during pregnancy and decreased after birth, and volume changes were associated with changes in TSH and BMI. They can be considered as indicators of metabolic and hemodynamic changes during pregnancy.

To assess the statistical significance of the relationship between thyroid volumes and laboratory performance levels (TSH, fT4 and fT3) during the first, second and third trimesters of pregnancy, a non-parametric analysis was performed, using the correlation coefficient and sig. (2-tailed). In the case of the

data for the first trimester, statistical significance of the relationship between the compared quantities was established. ($p < 0.05$): TSH ($p = 0.019$), fT4 ($p = 0.040$) and fT3 ($p = 0.028$). No statistical significance of association ($p > 0.05$) was found for the observed indicators for the second trimester: TSH ($p = 0.846$), fT4 ($p = 0.109$) and fT3 ($p = 0.698$) and third trimester TSH ($p = 0.945$), fT4 ($p = 0.394$) and fT3 ($p = 0.434$).

Population-specific reference TSH and fT4 reference in the 1, 2 and 3 trimesters of pregnancy respectively, in healthy pregnant women in the study population, intervals were determined from the values of the relevant indicators obtained so far. In calculating the TSH and fT4 reference intervals for each trimester of pregnancy, the parametric method was used. The data analysis was carried out by means of specialized software product RefVal 4,11. The criteria for the selection of the observed group are consistent with the generally accepted recommendations of the International Federation of Clinical Chemistry (IFCC) and the Institute of Clinical and Laboratory Standards (CLSI).

Reference intervals for TSH like those recommended by ATA for 2011 and BSE (2019) have also been obtained from some studies in Europe, with reference ranges predominantly based on the results of several studies in pregnant women. Other large-scale studies on center-specific and trimester-specific reference intervals have also been published. It is important to note that the results of these studies show significant population differences concerning the upper region of the TSH reference interval. These differences may be partly due on the one hand to differences in iodine status between populations and on the other hand to racial, geographical, and ethnic affiliation.

Our study found a specific reference interval for TSH for the first trimester 0.318–3.486 mIU/l. Close to ours are the results obtained from a study conducted in 2019 by Yanachkova et al. 0.30–2.97 mIU/l. Other authors get lower values for both lower and upper limit. The interval obtained by Haddow et al is 0.08–2.73 mIU/l. Similar results were obtained by Stricker et al. (0.09–2.83 mIU/l,) as well as Panesar et al. (0.03–2.30 mIU/l). In contrast, Marwaha et al. obtained higher scores (0.60–5.00 mIU/l).

For the second trimester, the reference interval for TSH we found is 0.947–4.897 mIU/l. Close to ours are the results obtained from a study conducted by Marwaha et al. (0.60–5.00 mIU/l). Other authors get lower values for both lower and upper limits. Such are the results obtained in 2019 by Yanachkova and Kol. (0.596–3.514 mIU/l). The one obtained by Soldin et al. range is 0.43–2.78 mIU/l,

also lower at both the lower and upper limits. Similar results are also of Stricker et al. (0.09–2.83 mIU/l) as well as Panesar et al. (0.03–2.30 mIU/l).

For the third trimester, the reference interval for TSH we found is 0.888–5.391 mIU/l. Close to ours are the results obtained from a study conducted by Marwaha et al. (0.74–5.70 mIU/l). Other authors get lower values for both lower and upper limits. Such are the results obtained in 2019 by Yanachkova and Kol. (1.647–3.870 mIU/l). The interval obtained by Soldin et al. is 0.43–2.78 mIU/l, it is also lower at both the lower tka and the upper limit. Similar results are also of Stricker et al. (0.31–2.90 mIU/l) as well as Panesar et al. (0.03–2.30 mIU/l).

The data from the analysis of the results obtained by the different authors show that the application of regional population-based reference limits for TSH will be most sensitive in the interpretation of TSH values and for the assessment of thyroid function in pregnant women.

In determining methodological and quarterly specific reference ranges for fT4, both physiological features related to pregnancy and the finding of a representative number of individuals with specific conditions such as pregnancy who are of normal iodine intake and from different ethnic groups have an influence. Despite the described difficulties in analyzing the concentration of fT4 during pregnancy, they also found differences from those in non-pregnant women. In one study by Lee RH et al. for example, it is reported that 18% (19/107) of the obtained fT4 values in the second trimester and 40% (27/68) in the third trimester are below, normal values for the non-pregnant population.

The reference intervals we obtained for the first 6.51–12.44 pmol/l, the second 4.26–11.45 pmol/l and the third 3.68–11.17 pmol/l trimester differ from those published by Gilbert et al., who found for the first trimester: 10.4–17.8 pmol/l, and for the second: 9–19 pmol/l. Similar are the results of Andersen et al. for the second trimester 10.30–18.15 pmol/l. Lican Han L. et al. in one 2018 study received for the first trimester 11.8–18.4 pmol/ L; for the second trimester 11.6–17.4 pmol/L; and for the third trimester respectively 9.7–15.1 pmol/L. Kostecka-Matyja M et al. in their 2017 study set out to assess trimester specific reference intervals for TSH, fT3 and fT4 for pregnant women in the Polish population. Their calculated reference values were 0.009–3.177, 0.05–3.442 and 0.11–3.53 mIU/L for TSH; 3.63–6.55, 3.29–5.45 and 3.1–5.37 pmol/L for fT3; and 11.99–21.89, 10.46–16.67 and 8.96–17.23 pmol/L for fT4 in consecutive quarters of pregnancy.

CONCLUSIONS

1. The results obtained on maternal age, familiarity for thyroid pathology, spontaneous pregnancy loss, infertility, and smoking in the observed group of pregnant women are comparable to data given in the literature studied.

2. The study of hormonal indicators of the thyroid gland found:
   - TSH values for the first trimester below a lower reference limit.
   - fT4 values for the first, second and third trimesters below a lower reference limit.

3. The observed slight increase in thyroid volumes in ml during pregnancy is within the physiological adaptation changes characteristic of this period.

4. A statistically significant direct correlation was found between thyroid volumes and levels of laboratory indicators (fT4 and fT3) in the first trimester of pregnancy and an inverse correlation between thyroid volumes and TSH levels for the same trimester.

5. The reference intervals of 0.318–3.486 mIU/l, 0.947–4.897 mIU/l and 0.888–5.391 mIU/l for TSH in the first, second and third trimesters, respectively, are different from the interval adopted for the non-pregnant population as well as from the values recommended by ATA and BSE.

6. Our constructed fT4 reference intervals in the first, second and third trimesters of 6.51–12.44 pmol/l, 4.26–11.45 pmol/l and 3.68–11.17 pmol/l respectively are different from those in the nonpregnant population.

CONTRIBUTIONS OF A SCIENTIFIC AND APPLIED NATURE

1. The study provides valuable data on physiological changes in the thyroid gland during pregnancy in women of our population.

2. The assessment of indicators of thyroid function, morphology and autoimmunity provides important information for the diagnosis of functional thyroid disorders and the need for therapy and follow-up of affected women.

3. Characterization of multiple thyroid-related variables will contribute to establishing reliable prognostic factors for thyroid dysfunction dynamics in the gestation period specific to our population.

4. These predictive factors could be used to produce mathematically multifactorial models to predict individual risk for gestational thyroid disorders.

5. These data would help to conduct targeted research on risk contingents and prepare reasoned recommendations for treatment and the frequency of follow-up.

6. The preparation and implementation of trimester-specific intervals for TSH, fT4 could help to:

   - Lowering the risk of incorrect interpretation of laboratory results and often hyperdiagnosis of thyroid dysfunction, which in turn is associated with significant risks for pregnancy and fetus.
   - Correct assessment of conditions of hypo- or hyperthyroidism which is of paramount importance to prevent complications of pregnancy.
   - Limiting the application of fixed TSH reference values for each trimester of pregnancy proposed in the consensus opinions of some authoritative thyroid organizations because, a major drawback in the use of fixed reference limits is the overdiagnosis of thyroid insufficiency found in some studies.

The preparation of a population-specific reference interval for TSH is a priority issue for endocrinology specialists, and studies in this direction are encouraged in the consensus opinions of all European and global thyroid organizations.

CONCLUSION

The described physiological processes determining the dynamics of TSH and thyroid hormones during pregnancy, the possibility of occurrence of specific complications and their prevention, as well as the risk of hyperdiagnostics, determine the need for laboratory assessment of gestational thyroid function to be carried out based on population-based trimester specific reference intervals. At present, the measurement of serum TSH remains the main factor in assessing the condition of the mother's thyroid gland and is used in decision-making and determining treatment goals. Since population reference intervals do not exist for our region and due to differences in the upper reference limit for TSH in pregnant women between populations, the determination of own trimester-specific reference ranges derived from the analysis of healthy, thyroid peroxidase antibodies (anti-TPO) and thyroglobulin antibodies (anti-Tg) negative pregnant women is of priority.

LIST OF SCIENTIFIC PUBLICATIONS, PARTICIPATION IN SCIENTIFIC FORUMS (INTERNATIONAL AND NATIONAL) AND PROJECTS ON THE TOPIC

Scientific publications:

Publications in scientific journals and collections referenced in Scopus and/or Web of Science:

Delev B, Deneva T. Nonchev B, Kilova K. Serum concentrations and trimester-specific reference intervals for TSH and fT4 during the last trimester of pregnancy. General Medicine, 2023, 25(4), 18-24

Publications in scientific journals and collections not refereed in Scopus and Web of Science:

Delev, B., Deneva, T., Nonchev, B., Argatska, A., Nyagolova, P., & Miteva M. Clinical laboratory evaluation of gestational thyroid function in the first and second trimester. Knowledge - International Journal, Vol. 47.4, pp 569–574.

Delev B, Deneva T, Nonchev B, Nyagolova P, Miteva M. Thyroid volume in healthy pregnant women from Plovdiv region. Knowledge – International Journal, Vol.55.4, pp. 575-580

Participation in scientific forums (international and national)

Boyan Delev, Tanya Deneva, Boyan Nonchev, Valya Markova "Dynamics in serum TSH levels during early pregnancy" 31st Annual Assembly of International Medical Association Bulgaria 28-31 October 2021. Medical University – Plovdiv.

Delev B, Deneva T, Nonchev B, Argatska A, Nyagolova P, Miteva M. Trimester specific reference intervals of TSH in healthy pregnant women from Plovdiv region - report. XIV National Conference on Clinical Laboratory, 14 – 16 October 2022, Grand Hotel Plovdiv

Projects:

Project No DPDP 21/2019 "Study of thyroid function in healthy pregnant women from the Plovdiv region and development of population-specific reference limits of thyroid stimulating hormone for each trimester of pregnancy."

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