

Exploration of Physiological Reference Intervals for Thyroid Function During the First Trimester of Twin Pregnancy

Xiangyu Liao¹, Xin Liang¹, Chongmin Li¹, Qi Shi¹, Kerong Zhang^{1*}

¹Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan Province, 637000, China

Corresponding author: Kerong Zhang, lxxy0918@163.com

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Abstract: Objective: To explore specific reference intervals for serum thyroid-stimulating hormone (TSH) and free thyroxine (FT4) during the first trimester of twin pregnancies in Nanchong City and compare them with those of singleton pregnancies.

Methods: A retrospective analysis was conducted on 788 twin pregnancies who underwent prenatal examination and delivery at the Affiliated Hospital of North Sichuan Medical College from January 2017 to May 2024. Based on the criteria established by the National Academy of Clinical Biochemistry (NACB), 184 cases were included in the twin pregnancy group. Additionally, 537 singleton pregnancies from the same hospital and time period were randomly selected as the singleton group, and 42 twin pregnancies admitted between June and December 2024 were assigned as the non-concurrent validation group. Reference intervals for early pregnancy thyroid hormones were established using the percentile method, with the 2.5th (P2.5) and 97.5th percentiles (P97.5) of TSH and FT4 in the twin pregnancy group set as the lower and upper limits of normal thyroid function. Mann–Whitney U tests were used to compare differences between groups. The non-concurrent group was used to validate the preliminary reference intervals. The diagnostic discrepancies in thyroid dysfunction under different criteria were also analyzed.

Results: The TSH reference intervals in twin pregnancies during the first trimester were as follows: overall (T1) 0.005–4.28 mIU/L (median 1.15); gestational weeks 1–6+6 (T1-1) 0.7–4.58 mIU/L (median 1.87); weeks 7–13+6 (T1-2) 0.004–4.11 mIU/L (median 0.78). FT4 reference intervals were: T1 0.98–2.60 ng/dl (median 1.29); T1-1 1.0–1.86 ng/dl (median 1.24); T1-2 0.97–2.68 ng/dl (median 1.32). Compared to singleton pregnancies, twin pregnancies exhibited significantly lower TSH levels (1.15 vs. 1.56 mIU/L, $Z = -3.909$, $P < 0.01$) and higher FT4 levels (1.29 vs. 1.27 ng/dl, $Z = -2.882$, $P = 0.003$) in the first trimester. These differences were more pronounced during T1-2 (TSH: 0.78 vs. 1.25 mIU/L, $Z = -3.723$, $P < 0.001$; FT4: 1.32 vs. 1.25 ng/dl, $Z = -4.219$, $P < 0.01$). Using singleton-based reference intervals or the 2017 American Thyroid Association (ATA) guidelines resulted in a significant overdiagnosis of subclinical hyperthyroidism in twin pregnancies ($\chi^2 = 34.833a$, $\chi^2 = 31.233a$, $P < 0.01$). However, applying twin-specific reference intervals reduced the diagnosis of low FT4 syndrome compared to the ATA 2017 criteria ($\chi^2 = 6.715a$, $P = 0.01$).

Conclusion:Thyroid function indices in twin pregnancies during early gestation in Nanchong City display distinct dynamic characteristics compared to singleton pregnancies. Establishing twin-specific reference intervals is crucial. Applying singleton-based or international guideline thresholds may lead to misdiagnosis and inappropriate treatment in twin pregnancies, posing risks to maternal and fetal health. Therefore, clinical assessment should preferentially adopt population-specific reference standards tailored for twin pregnancies.

Keywords:Twin pregnancy; reference interval; thyroid hormones

Introduction

Thyroid dysfunction during pregnancy has been confirmed to be associated with adverse pregnancy outcomes, including miscarriage, preterm birth, and preeclampsia ^[1-2]. Both elevated and decreased maternal free thyroxine (FT4) levels may negatively affect fetal brain development ^[3]. Given the detrimental association between thyroid dysfunction and adverse neonatal outcomes, timely and appropriate pharmacologic intervention is of critical importance. However, studies have shown that excessive thyroid hormone replacement therapy may lead to neurodevelopmental impairments in children and has also been linked to an increased risk of small-for-gestational-age infants and preterm birth ^[4-5]. Moreover, inappropriate use of antithyroid drugs may damage maternal leukocyte and liver function and even cause fetal malformations ^[6]. Therefore, accurate identification of thyroid dysfunction and avoidance of inappropriate pharmacologic interventions are essential for safeguarding maternal and fetal health.

According to the 2017 American Thyroid Association (ATA) Guidelines ^[7], due to differences in ethnicity, geographic location, and iodine nutrition status, it is necessary for each region to establish trimester-specific reference ranges for thyroid function during pregnancy. In the absence of locally derived reference intervals, a TSH upper limit of 4.0 mIU/L is recommended. Similarly, the second edition of the “Guidelines for the Diagnosis and Treatment of Thyroid Diseases During Pregnancy and Postpartum” published in China in 2019 ^[8] strongly advocates for the establishment of region-specific reference ranges. In recent years, numerous clinical studies have been conducted both domestically and internationally, but most have focused on singleton pregnancies. Research on twin pregnancies remains limited. However, it is known that serum human chorionic gonadotropin (hCG) levels in women with twin pregnancies are significantly higher and remain elevated for longer periods compared to singleton pregnancies ^[9].

Grun et al . ^[10] reported that the incidence of suppressed TSH levels in twin pregnancies is three times that observed in singleton pregnancies. These findings suggest that the patterns of thyroid hormone changes during pregnancy may differ between singleton and twin pregnancies, and reference intervals derived from singleton pregnancies may not be applicable to women with twin gestations.

This study aims to establish trimester-specific reference intervals for thy-

roid function in women with twin pregnancies in the Nanchong region and to investigate the prevalence of thyroid function abnormalities during the first trimester. The findings are intended to provide evidence for standardized diagnosis and management of thyroid disorders in women with twin pregnancies. Previous research ^[11] has indicated that Sichuan Province is an iodine-sufficient region. A study by Mata ^[12] also showed that mild iodine deficiency does not significantly alter thyroid hormone reference limits in pregnant women.

1. Materials and Methods

1.1 Study Population

A total of 788 women with twin pregnancies who received prenatal care and delivered at the Affiliated Hospital of North Sichuan Medical College between January 2017 and May 2024 were initially recruited. After applying the inclusion and exclusion criteria, 184 participants meeting the criteria for “standard population” as defined by the National Academy of Clinical Biochemistry (NACB) were included as the twin pregnancy group. In addition, 736 singleton pregnant women who received prenatal care and delivered during the same period were randomly selected at a 1:4 ratio. After applying the same criteria as for the twin group, 537 women were included in the singleton group. Furthermore, 42 twin pregnancies from June 2024 to December 2024 were enrolled as a non-contemporaneous comparison group.

This study protocol was reviewed and approved by the Biomedical Ethics Committee of the Affiliated Hospital of North Sichuan Medical College (Approval No. 2024ER687-1).

1.2 Inclusion and Exclusion Criteria

1.2.1 Twin Pregnancy Group – Standard Population

Participants in the twin group were selected based on NACB recommendations for defining a standard population.

Inclusion Criteria:

- (1) Continuous residence in Nanchong for ≥ 2 years;
- (2) Aged 18–40 years;
- (3) Twin pregnancy with two live births at delivery;
- (4) Han ethnicity.

Exclusion Criteria:

- (1) Conception via assisted reproductive technology;
- (2) History or family history of thyroid disease prior to pregnancy;
- (3) Use of medications affecting thyroid function (e.g., amiodarone, lithium, antipsychotics, anticonvulsants, levothyroxine);
- (4) Absence of thyroid function testing during the first trimester or tests not performed at our institution;
- (5) Abnormal thyroid morphology detected by clinical palpation or ultra-

sound;

- (6) Positive thyroid autoantibodies (TPOAb and/or TgAb);
- (7) Presence of major organ dysfunction or immune-metabolic disorders (e.g., cardiovascular, endocrine, rheumatic diseases, malignancies);
- (8) Severe hyperemesis gravidarum during pregnancy.

1.2.2 Singleton Pregnancy Group

Inclusion and exclusion criteria were identical to those used for the twin pregnancy group, except that the participants had singleton pregnancies.

1.3 Research Methods

1.3.1 Data Collection

Data were extracted from the hospital's integrated database (outpatient, inpatient, and delivery records), including maternal age, height, weight, pre-pregnancy body mass index (BMI), gravidity, parity, residence, medical and family history, medication use, and gestational age at the time of thyroid function testing.

1.3.2 Grouping

From the 788 initially enrolled twin pregnancies, 184 women were included in the standard population-based twin pregnancy group after screening. Using a 1:4 random sampling ratio, 736 singleton pregnancies were selected, and 537 cases remained after applying exclusion criteria, forming the singleton group. An additional 42 twin pregnancies collected from June to December 2024 formed the non-contemporaneous twin group. Gestational stages for all groups were classified based on the 10th edition of Obstetrics and Gynecology (People's Medical Publishing House), with early pregnancy (T1) defined as gestational age 1–13+6 weeks. This was further subdivided into T1-1 (1–6+6 weeks) and T1-2 (7–13+6 weeks).

1.3.3 Laboratory Testing

Participants fasted for at least 8 hours prior to blood sampling. In the morning, 3–5 mL of venous blood was collected from the antecubital vein by trained personnel in the hospital's laboratory. After standardized pre-processing, samples were immediately transported to the Department of Nuclear Medicine. Serum thyroid function indicators, including TSH, FT4, and thyroid autoantibodies (TPOAb, TgAb), were quantitatively assessed using chemiluminescent immunoassay (CLIA) on the Siemens ADVIA Centaur XP automated analyzer. Calibration and quality control were performed using manufacturer-provided reagents, and all procedures followed strict standard operating and quality control protocols.

1.3.4 Definitions

Pre-pregnancy BMI was calculated as weight (kg) divided by height squared (m²). Based on the "Guidelines for Prevention and Control of Over-

weight and Obesity in Chinese Adults”^[13], BMI <18.5 kg/m² was classified as underweight; 18.5–23.9 kg/m² as normal weight; 24.0–27.9 kg/m² as overweight; and ≥28.0 kg/m² as obese.

1.3.5 Diagnostic Criteria for Thyroid Dysfunction

The reference intervals for thyroid function during early pregnancy (serum TSH and FT4) were determined using the 2.5th–97.5th percentile (P2.5–P97.5). Based on the reference intervals established in the singleton and twin groups, thyroid dysfunction was classified as follows:

- Overt hyperthyroidism: TSH below the lower limit and FT4 above the upper limit.
- Subclinical hyperthyroidism: TSH below the lower limit with FT4 within the reference range.
- Overt hypothyroidism: TSH above the upper limit and FT4 below the lower limit.
- Subclinical hypothyroidism: TSH above the upper limit with FT4 within the reference range.
- Isolated hypothyroxinemia: FT4 below the lower limit with normal TSH levels.
- Positive thyroid autoantibodies (TPOAb/TgAb): Antibody levels exceeding the manufacturer’s recommended upper limit.

According to the 2017 ATA Guidelines^[7], trimester-specific TSH reference ranges are: 0.1–4.0 mIU/L for the first trimester, 0.2–4.0 mIU/L for the second trimester, and 0.3–4.0 mIU/L for the third trimester. FT4 reference intervals were determined based on the assay kit used in the hospital’s Department of Nuclear Medicine: 1.11–1.59 ng/dL for the first trimester, 0.97–1.46 ng/dL for the second trimester, and 0.89–1.36 ng/dL for the third trimester.

1.3.6 Reference Interval Validation

A small-sample validation method was employed to assess the reliability of the self-established reference intervals for thyroid function during early pregnancy in twin pregnancies. The validation was considered successful if more than 90% of test results fell within the established reference interval.

1.4 Statistical Analysis

All statistical analyses were performed using SPSS version 27.0. Reference intervals for biological indicators were constructed using the non-parametric method to determine the 95% confidence intervals (95% CI). The Kolmogorov–Smirnov test was used to evaluate the distribution of continuous variables. Data conforming to a normal distribution were expressed as mean ± standard deviation, and differences between groups were assessed using the independent samples t-test. For non-normally distributed data, results were expressed as medians with interquartile ranges (IQRs), and the Mann–Whitney U test was

applied for between-group comparisons. Categorical variables were presented as frequencies (percentages), and intergroup associations were evaluated using the chi-square (χ^2) test. A two-tailed P value of <0.05 was considered statistically significant.

2. Results

2.1 Baseline Characteristics

A total of 788 twin pregnancies were initially enrolled in the study. After applying the exclusion criteria, 463 cases conceived via assisted reproductive technology, 10 with a history of thyroid disease prior to pregnancy, 8 who received antithyroid treatment during pregnancy, 36 with positive TPOAb and/or TgAb results, 73 with missing or externally tested early pregnancy thyroid function data, and 14 with other significant comorbidities (including pre-pregnancy hypertension, diabetes mellitus, or autoimmune diseases) were excluded. Finally, 184 twin pregnancies were included in the twin group. Concurrently, 537 singleton pregnancies were included as the singleton group.

There were no statistically significant differences between the two groups in terms of maternal age, proportion of primiparas, or median gestational age at thyroid function testing during each trimester ($P > 0.05$) (see **Table 1**).

Table 1. Comparison of Baseline Characteristics Between Twin and Singleton Pregnancy Groups

Group	n	Maternal Age (years)	Pre-pregnancy BMI (kg/m ²)	Primiparous n (%)	Gestational Age at T1 Testing (weeks)	Gestational Age at T1-1 Testing (weeks)	Gestational Age at T1-2 Testing (weeks)
Singleton Pregnancy	537	29.5(27,33)	20.8(19.2,22.8)	369(68.72)	8.5(6.4,11.9)	5.5(4.6,6.3)	10.3(8.4,12.4)
Twin Pregnancy	184	29(27,32)	22.0(20.2,24.4)	136(73.91)	9.5(7,12.2)	5.5(4.6,6.0)	11(8.5,12.4)
Z (or χ^2) Value		-1.096	-4.495	1.763a	-1.832	-0.877	-0.712
P-value		0.273	< 0.05	0.184	0.067	0.3881	0.476

Note: a indicates the χ^2 value. Gestational age at testing refers to the median gestational age at which maternal thyroid function was assessed.

2.2 Reference Intervals for Serum TSH and FT4 in Twin Pregnancies during the First Trimester

The reference intervals for serum TSH in twin pregnancies during early gestation were as follows:

- Overall first trimester (T1): 0.005–4.28 mIU/L (median: 1.15)
- Gestational weeks 1–6+6 (T1-1): 0.7–4.58 mIU/L (median: 1.87)
- Gestational weeks 7–13+6 (T1-2): 0.004–4.11 mIU/L (median: 0.78)

The reference intervals for FT4 were:

- T1: 0.98–2.60 ng/dL (median: 1.29)
- T1-1: 1.00–1.86 ng/dL (median: 1.24)
- T1-2: 0.97–2.68 ng/dL (median: 1.32)

(See Table 2)

2.3 Comparison of Serum TSH and FT4 between T1-1 and T1-2 in Twin Pregnancies

A comparison of serum TSH and FT4 levels between the T1-1 group (n = 46) and T1-2 group (n = 138) showed that the median TSH level in T1-2 was significantly lower than in T1-1 (0.78 vs. 1.87 mIU/L, $P < 0.001$). Conversely, the median FT4 level in T1-2 was significantly higher than in T1-1 (1.32 vs. 1.24 ng/dL, $P = 0.006$). These differences were statistically significant. (See Table 2)

2.4 Reference Intervals for Serum TSH and FT4 in Singleton Pregnancies during the First Trimester

The reference intervals for serum TSH in singleton pregnancies during early gestation were as follows:

- Overall T1: 0.19–4.48 mIU/L (median: 1.56)
- T1-1: 0.42–4.56 mIU/L (median: 1.90)
- T1-2: 0.06–3.91 mIU/L (median: 1.25)

The reference intervals for FT4 were:

- T1: 0.97–1.62 ng/dL (median: 1.27)
- T1-1: 1.00–1.61 ng/dL (median: 1.28)
- T1-2: 0.96–1.62 ng/dL (median: 1.25)

(See Table 2)

2.5 Comparison of Serum TSH and FT4 Levels between Twin and Singleton Pregnancies during Early Gestation

Compared to the singleton pregnancy group, the median TSH level in the twin pregnancy group during the overall first trimester was significantly lower (1.15 vs. 1.56 mIU/L, $P < 0.01$), while the FT4 level showed an upward trend (1.29 vs. 1.27 ng/dL, $P = 0.004$).

In the T1-1 subgroup, TSH and FT4 levels in singleton pregnancies were slightly higher than in twin pregnancies; however, the differences were not statistically significant (TSH: 1.90 vs. 1.87 mIU/L, $P = 0.925$; FT4: 1.28 vs. 1.24 ng/dL, $P = 0.179$).

In the T1-2 subgroup, the median TSH level in twin pregnancies was significantly lower than that in singleton pregnancies (0.78 vs. 1.25 mIU/L, $P < 0.001$), while the median FT4 level was significantly higher (1.32 vs. 1.25 ng/dL, $P < 0.01$). (See Table 2)

Table 2. Comparison of Serum TSH and FT4 in Early Pregnancy between Twin and Singleton Groups [Median (P2.5, P97.5)]

Indicator	Gestational Period	Twin Group M (P2.5, P97.5) (Sample Size)	Singleton Group M (P2.5, P97.5) (Sample Size)	Z Value	P Value
TSH(mIU/L)	T1	1.15(0.005,4.28) (184)	1.56(0.19,4.48) (537)	-3.909	< 0.01
	T1-1	1.87(0.70,4.58) (46)	1.90(0.42,4.56) (232)	-0.094	0.925
	T1-2	0.78 (0.004,4.11)(138)	1.25(0.06,3.91) (305)	-3.723	< 0.001
FT4(ng/dl)	T1	1.29(0.98,2.60) (184)	1.27(0.97,1.62) (537)	-2.882	0.004
	T1-1	1.24(1.00,1.86) (46)	1.28(1.00,1.61) (232)	-1.342	0.179
	T1-2	1.32 (0.97,2.68) (138)	1.25(0.96,1.62) (305)	-4.219	< 0.01

Note: TSH – Thyroid Stimulating Hormone; FT4 – Free Thyroxine.

2.6 Validation of the Established Reference Intervals for the Twin Pregnancy Group Using a Small Sample Verification Method

A total of 42 twin pregnant women from a non-concurrent cohort were selected for validation. The verification results for TSH and FT4 at each gestational stage showed that over 90% of the data fell within the newly established reference intervals, indicating that the validation was effective. See **Table 3**.

Table 3. Validation Results of Thyroid Function Reference Intervals at Different Gestational Stages in the Non-Concurrent Twin Pregnancy Group

Indicators	Gestational Stage	Established Reference Interval	Validation Population Interval	Validation Results Positive Rate Negative Rate	
TSH(mIU/L)	T1	0.005~4.28	0.05~4.16	0%	100%
	T1-1	0.70~4.58	0.93~4.19	0%	100%
	T1-2	0.004~4.11	0.03~2.81	0%	100%
FT4(ng/dl)	T1	0.98~2.59	0.89~1.76	5%	95%
	T1-1	1.0~1.86	0.94~1.67	10%	90%
	T1-2	0.97~2.68	0.94~1.99	5%	95%

2.7 Detection of Thyroid Dysfunction in Twin Pregnancies During the First Trimester Using Different Reference Intervals

In the twin pregnancy cohort, the detection rates of thyroid dysfunction based on the newly established twin-specific reference intervals during the first trimester (T1) were as follows: overt hypothyroidism 0%, subclinical hypothyroidism 2.2%, hyperthyroidism 1.6%, and low FT4 syndrome 2.2%. Using the singleton-specific T1 reference intervals, the corresponding rates were 0%, 1.6%, 21.2%, and 1.1%, respectively. According to the 2017 American Thyroid Association (ATA) guidelines, the rates were 0%, 5.4%, 19.6%, and 8.2%, respectively.

When compared with the twin-specific reference intervals, both the singleton-specific intervals and the ATA guidelines showed significantly higher detection rates of hyperthyroidism, with statistically significant differences ($\chi^2 = 34.833a$, $P < 0.01$; $\chi^2 = 31.233a$, $P < 0.01$). Additionally, the incidence of low FT4 syndrome was significantly lower when using the twin-specific reference intervals compared to the ATA standards ($\chi^2 = 6.715a$, $P = 0.01$). These findings

suggest that reference intervals tailored to twin pregnancies may reduce misclassification of thyroid dysfunction. See **Table 4** for details.

Table 4. Distribution of Thyroid Dysfunction Diagnosed by Different Criteria in Twin Pregnancies During the First Trimester [N = 184, n (%)]

Reference Criteria	Hyperthyroidism	Overt Hypothyroidism	Subclinical Hypothyroidism	Low FT4 Syndrome
Self-established reference interval for twin pregnancies during the first trimester (T1)	3(1.6)	0	4(2.2)	4(2.2)
Self-established reference interval for singleton pregnancies during the first trimester (T1)	39(21.2)	0	3(1.6)	2(1.1)
2017 ATA Guidelines	36(19.6)	0	10(5.4)	15(8.2)
χ^2 a	34.833a	-	-	-
χ^2 b	31.233a	-	2.673a	6.715a
P1	P < 0.01	-	1.0	0.685
P2	P < 0.01	-	0.102	0.01

Note: a indicates the chi-square value; $\chi^2(a)$ and P₁ represent the chi-square value and P-value for the comparison between the self-established reference intervals for twin and singleton groups;

$\chi^2(b)$ and P₂ represent the chi-square value and P-value for the comparison between the twin group's reference interval and the 2017 ATA guideline.

2.8 Distribution of Thyroid Dysfunction in Twin Pregnant Women During the T1-1 Period According to Different Diagnostic Criteria

When using the reference interval for the overall T1 period as the standard, the detection rate of subclinical hypothyroidism was higher compared to using the T1-1 period reference interval, while the detection rates of hyperthyroidism and low FT4 syndrome were slightly lower. However, none of these differences reached statistical significance ($P > 0.05$). See **Table 5**.

Table 5. Distribution of Thyroid Dysfunction in Twin Pregnant Women During the T1-1 Period According to Different Diagnostic Criteria [N = 46, n (%)]

Reference Criteria	Hyperthyroidism	Overt Hypothyroidism	Subclinical Hypothyroidism	Low FT4 Syndrome
Self-established twin pregnancy T1 period reference interval as the standard	0(0)	0(0)	3(6.5)	0(0)
Self-established twin pregnancy T1-1 period reference interval as the standard	1(2.2)	0(0)	1(2.2)	1(2.2)
P	1.0	-	0.617	1.0

Note: $P < 0.05$ indicates a statistically significant difference.

2.9 Distribution of Thyroid Dysfunction in Twin Pregnant Women at T1-2 Period According to Different Diagnostic Criteria

Compared with the reference interval at T1 period, using the T1-2 period

reference interval as the standard showed a higher detection rate of subclinical hypothyroidism and a lower detection rate of low FT4 syndrome. No significant difference was observed in hyperthyroidism between the two groups. All differences were not statistically significant ($P > 0.05$). See **Table 6**.

Table 6 Distribution of Thyroid Dysfunction in Twin Pregnant Women at T1-2 Period According to Different Diagnostic Criteria[N=138, n (%)]

Reference Criteria	Hyperthyroidism	Overt Hypothyroidism	Subclinical Hypothyroidism	Low FT4 Syndrome
Self-established twin pregnancy T1 period reference interval as the standard	4(2.3)	0	1(0.7)	4(2.9)
Self-established twin pregnancy T1-2 period reference interval as the standard	3(2.2)	0	4(3.6)	2(1.4)
P	1.0	-	0.37	0.684

Note: $P < 0.05$ indicates a statistically significant difference.

3 Discussion

With the increasing proportion of advanced maternal age and the widespread use of assisted reproductive technologies, the incidence of twin pregnancies has risen annually^[14]. Compared with singleton pregnancies, twin pregnancies carry higher perinatal risks; women with twin pregnancies are more prone to miscarriage, preterm birth, gestational hypertension, and fetuses have a higher risk of growth restriction^[15]. The fetal thyroid begins synthesizing thyroid hormones (TH) at 10–12 weeks of gestation, but thyroid function is not fully developed until 18–20 weeks; during early pregnancy, the fetus primarily depends on maternal serum thyroid hormones^[16–17]. As a special pregnancy type, twin pregnancies show more pronounced thyroid hormone fluctuations than singletons to ensure adequate TH supply for both the mother and developing fetuses^[18–19].

During early pregnancy, human chorionic gonadotropin (hCG), secreted by the placenta as a pregnancy-specific marker, shares homology with the α -subunit of the thyroid-stimulating hormone (TSH) receptor, conferring thyrotropic activity^[20]. Studies have shown that for every 10,000 U/L increase in hCG concentration, TSH levels decrease by approximately 0.1 mIU/L, with this dose-dependent effect being most pronounced at the hCG peak between 8–10 weeks of gestation^[9]. Due to increased placental volume, hCG secretion in twin pregnancies is higher and prolonged compared to singletons, which continuously stimulates thyroid secretion of free thyroxine (FT4) via TSH receptor pathways while more strongly suppressing pituitary TSH synthesis^[10]. Zhang et al.^[21] analyzed thyroid function in 317 women with twin pregnancies in Tianjin and found that TSH levels gradually declined to a nadir after 8 weeks gestation, then rose, remaining lower and for a longer duration than in singleton pregnancies. Zhang Chao et al.^[22] established a TSH reference interval of 0.03–3.99 mIU/L for 352 twin pregnancies, noting early pregnancy TSH levels higher than in singletons.

Jiang et al.^[23] evaluated thyroid function in 160 twin pregnancies early in gestation and found a TSH range of 0.01–3.35 mIU/L, with median TSH lower and median FT4 higher than singletons. Ashoor et al.^[24], through a prospective study, established thyroid hormone reference ranges at 11–13 weeks for twin pregnancies; in TPO antibody-negative women with normal outcomes, serum TSH median multiples were lower compared to singletons, with no significant difference in FT4.

Our study's self-established twin pregnancy reference interval for early pregnancy showed an upper TSH limit of 0.005–4.28 mIU/L, with median TSH significantly lower in twins than singletons (1.15 vs. 1.56 mIU/L), and FT4 levels slightly higher (1.29 vs. 1.27 ng/dL). Although our TSH upper limit differs somewhat from previous studies, the trend of lower TSH in twin pregnancies compared to singletons aligns with earlier findings. Differences in FT4 results compared to Ashoor et al. may relate to sample size, ethnicity, geography, assay kits, and iodine nutritional status. Additionally, a large longitudinal Chinese study^[25] demonstrated gestational age-related changes in thyroid function: TSH levels were similar to non-pregnant states at 4–6 weeks, then sharply decreased to a nadir at 7–12 weeks. Jiang et al.^[23] also confirmed this pattern in early pregnancy sub-stages. Our study found that the overall upper limit of TSH in early pregnancy was higher than the American Thyroid Association (ATA) guideline for singletons (4.0 mIU/L)^[7], but the 7–13+6 weeks gestational TSH upper limit (4.11 mIU/L) was close. The relatively higher upper limit before 7 weeks (4.58 mIU/L) and small sample size may have elevated the overall reference range, highlighting the importance of establishing specific reference intervals for twin pregnancies and considering sub-stage stratification in early pregnancy.

The 2022 Chinese guideline on thyroid disease management during pregnancy^[26] reported prevalence rates of clinical hypothyroidism (CH) in pregnant women at 0.3–1.0%, subclinical hypothyroidism (SCH) at 4.0–17.8%, and thyroid toxicosis at approximately 1.0%. Some studies on twin pregnancies^[22,27] reported early pregnancy hyperthyroidism detection rates of 0–1.1%, hypothyroidism rates of 0.5–3.5%, and subclinical hypothyroidism at 1.8%. In this study, using the self-established twin pregnancy early pregnancy reference interval as standard, the subclinical hyperthyroidism detection rate was 1.7%, significantly lower than rates based on the self-established singleton interval and the 2017 ATA guideline (21.2% and 19.6%, respectively), but consistent with twin pregnancy reports (0–1.1%). Notably, using singleton or ATA standards could lead to about 12% of twin pregnancies being overdiagnosed with hyperthyroidism, consistent with Song Qinfeng et al.'s report^[27] where ~18% of twin pregnancies were overdiagnosed using singleton criteria. Such misdiagnosis increases psychological burden and may prompt unnecessary interventions. In our study, clinical hypothyroidism detection was 0%, and subclinical hypothyroidism was 2.2%, lower than the 4.0–17.8% reported in the 2022 Chinese Thyroid Association guideline but comparable to twin pregnancy studies (0.5–3.5%, 1.8%). This may

be due to our wider upper limit reference range for twins compared to the ATA guideline, possibly reducing false positives. Additionally, twin pregnancies have higher and prolonged hCG secretion compared to singletons, leading to more pronounced TSH suppression, which may partially compensate for thyroid insufficiency and delay progression of subclinical hypothyroidism^[7]. Furthermore, Li et al. demonstrated that TSH levels vary with gestational age^[25]. Therefore, this study further characterized the detection rates of thyroid dysfunction in twin pregnancies during the T1-1 and T1-2 subphases. Compared to using the overall T1 reference interval as the standard, establishing specific reference intervals for T1-1 and T1-2 phases in early pregnancy may reduce both underdiagnosis and misdiagnosis. However, due to the relatively small sample size and low detection rates in this study, further large-scale, multicenter investigations are warranted.

Thyroid function indicators in early pregnancy among twin pregnancies in the Nanchong region may exhibit distinct dynamic changes compared to singleton pregnancies. Utilizing current reference intervals designed for singleton pregnancies or international guideline standards may result in inaccurate thyroid function assessment in twin pregnancies. Establishing region-specific thyroid hormone reference intervals for twin pregnancies could enable more precise diagnosis, standardized management, and reduce diagnostic errors. Additionally, as thyroid hormone levels may vary with gestational age, constructing gestational week-specific reference intervals should be considered.

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