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Sex and Age Specific Hormonal Patterns in Healthy Chinese Individuals: A Cross-Sectional Study of Reproductive Hormones.

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Abstract

Background: Hormonal levels change with age and impact key physiological functions. However, limited data exist on age- and sex-specific hormone reference intervals in Asian populations.This study aimed to establish normative ranges for reproductive hormones in healthy Chinese adults to support age-adjusted clinical evaluations.

Methods: A cross-sectional sample of 500 healthy individuals (250 males, 250 females), aged 19–70 years, was recruited from the blood transfusion department of The Affiliated Hospital of Yangzhou University.Serum samples were collected under standardized fasting and resting conditions.Steroid

hormones (testosterone, estradiol, progesterone) were measured using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), while protein hormones (LH, FSH, prolactin) were analyzed using chemiluminescent immunoassay (CLIA). Reference intervals were derived based on IFCC guidelines.Statistical analyses included ANOVA, post-hoc Tukey tests, and t-tests.

Results: In males, testosterone showed a progressive decline with age (6.0 → 3.5 ng/mL, $p < 0.001$), while estradiol declined modestly (35 → 25 pg/mL).Progesterone decreased gradually (0.30 → 0.15 ng/mL, $p < 0.001$). LH and FSH increased significantly (4.5 → 9.5 mIU/mL and 4.0 → 16.0 mIU/mL, $p < 0.001$), whereas prolactin remained stable (~8.7 ng/mL).In females, testosterone levels were lower overall (0.40 → 0.18 ng/mL), with sharp postmenopausal declines in estradiol (110 → 20 pg/mL) and progesterone (3.5 → 0.2 ng/mL).LH and FSH rose markedly after menopause (6.5 → 32.0 mIU/mL; 7.0 → 70.0 mIU/mL, $p < 0.001$).Prolactin remained consistent across all ages (~12.7 ng/mL).

Conclusion: This study provides sex- and age-specific hormone reference intervals for a healthy Chinese population, highlighting endocrine aging patterns and the need for age-adjusted assessment.The gradual decline of progesterone in males, of uncertain clinical significance, is noted.

Key words: Hormonal Aging, Age-Specific Reference Ranges, Testosterone Decline, Luteinizing Hormone (LH), Progesterone Reduction, Prolactin Stability, Cross-Sectional Analysis.

Highlights:

- First comprehensive age-stratified reference intervals for six reproductive hormones (testosterone, estradiol, progesterone, LH, FSH, prolactin) in healthy Chinese adults, utilizing gold-standard LC-MS/MS for steroid hormones and CLIA for protein hormones.
- Demonstrates sex-divergent aging patterns: males show progressive testosterone decline (6.0 → 3.5 ng/mL, $p < 0.001$), while females exhibit

sharp postmenopausal reductions in estradiol (110 → 20 pg/mL) and progesterone (3.5 → 0.2 ng/mL).

- Reveals significant gonadotropin surges post-menopause (FSH: 7 → 70 mIU/mL; LH: 6.5 → 32 mIU/mL), contrasting with stable prolactin levels across all ages.
- Identifies a gradual progesterone decline in aging males (0.30 → 0.15 ng/mL, $p < 0.001$)—a novel finding with potential neuroendocrine implications.
- Establishes ethnically relevant reference intervals addressing long-standing data gaps in Asian populations and aiding age-adjusted endocrine assessment.

Plain English Summary

Hormones are chemical messengers that regulate vital functions such as reproduction, mood, energy, and bone health. As people age, hormone levels change naturally—but doctors often lack clear reference values specific to Asian populations.This study measured key reproductive hormones in 500 healthy Chinese men and women (aged 19–70 years) to understand how these levels typically change with age.

What we found:

- Men show a gradual decline in testosterone with age, which is associated (but not necessarily caused) with changes in energy, muscle strength, and sexual health.
- Women experience sharp drops in estrogen (estradiol) and progesterone after menopause, increasing risk factors for bone loss and metabolic changes.
- Both sexes display rising levels of LH and FSH after middle age, indicating the body’s natural reproductive aging process.
- Prolactin levels remain stable across all ages, meaning abnormal prolactin usually signals illness, not normal aging.

Why it matters: These results provide reliable, population-specific reference ranges for Chinese adults helping doctors:

- Differentiate normal hormonal aging from true endocrine disorders
- Avoid unnecessary hormone treatments (e.g., testosterone therapy in healthy older men)
- Improve diagnostic accuracy for Asian patients by using ethnicity-appropriate benchmarks

Future research will track individuals over time to personalize hormone health assessment and refine diagnostic thresholds for age-related hormonal changes.

Introduction

The establishment of age- and sex-specific reference intervals for reproductive hormones is fundamental for the accurate clinical assessment of endocrine function.Key hormones such as testosterone, estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) have well-defined roles in gonadal function and serve as critical markers for diagnosing endocrine disorders [1].Their levels are known to change with age; for instance, estradiol declines sharply in postmenopausal women, while LH and FSH typically rise in response to diminished gonadal feedback, patterns consistently observed in aging populations [1, 2]. Establishing reliable reference ranges for these hormones in healthy populations is therefore crucial for distinguishing normal aging from pathological conditions[1, 2].However, a significant challenge in applying existing normative data globally is that much of it is derived from Western populations.The generalization of these reference ranges to Asian cohorts, where ethnic and methodological variations may lead to different ranges, poses a risk of clinical misdiagnosis[3].This is particularly evident for testosterone in men, where large-scale studies of Chinese men using immunoassays have reported stable

or even rising levels with age [3, 4], contradicting the conventional narrative of a universal decline and highlighting a critical gap in population-specific data.These discrepancies underscore the limitations of steroid immunoassays, which are prone to cross-reactivity and may lack the accuracy needed for precise hormonal quantification[5, 6].

This study aims to address this gap by establishing robust, age-specific reference intervals for a healthy Chinese cohort.To ensure high methodological accuracy, we utilized Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) for steroid hormone quantification, thereby minimizing the cross-reactivity issues inherent in steroid immunoassays [5, 6]. We focused on a core panel of hormones with clear clinical utility: testosterone and estradiol for gonadal function, and LH and FSH as pituitary feedback markers.Prolactin was included as a stable biomarker where deviations typically indicate pathology rather than normal aging[7]. Progesterone was also measured for a comprehensive profile, though its clinical significance in basal states, particularly in men, is less defined[8].By analyzing a cross-sectional sample of 500 healthy Chinese individuals stratified by age and sex, this study provides foundational data to aid clinicians in making accurate, age-adjusted assessments and in distinguishing physiological changes from pathological states in this demographic.

Materials and Methods

Study Design and Population

This cross-sectional study included 500 healthy Chinese individuals (250 males, 250 females) aged 19--70 years.Participants were recruited from the blood transfusion department (comprising healthy blood donors) of The Affiliated Hospital of Yangzhou University.It is important to note that while blood donors represent a generally healthy segment of the population, normal reproductive function was not directly verified, representing a potential

limitation in establishing a reference range for reproductive health[5].All procedures involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.The study also adhered to national guidelines for clinical research in China.

Of 568 volunteers screened, 68 (12%) were excluded for medication use (n=23), irregular menses (n=18), abnormal BMI (n=17), or recent illness (n=10). Healthy individuals were defined as those without self-reported endocrine, metabolic, cardiovascular, renal (eGFR ≥60 mL/min/1.73 m^2), or chronic diseases, and with a body mass index (BMI) <30 kg/m^2.Premenopausal women (aged 19--45 years) were required to have normal menarche and regular menstrual cycles (21--35 days).

Inclusion and Exclusion Criteria

Inclusion Criteria:

① Healthy individuals aged 19--70 years. ② No self-reported history of endocrine, metabolic, cardiovascular, or chronic diseases. ③ No history of hormonal therapy use within the past six months. ④ Female participants were categorized based on their reproductive status (premenopausal, perimenopausal, postmenopausal).

Exclusion Criteria:

① Individuals with diagnosed endocrine disorders (e.g., hypogonadism, PCOS, thyroid dysfunction). ② History of hormone replacement therapy, oral contraceptives, or corticosteroid use within the last six months. ③ Participants with chronic illnesses such as diabetes, hypertension, cardiovascular disease, liver or kidney dysfunction. ④ Women who were pregnant or lactating. ⑤ Participants reporting smoking (>5 cigarettes/day), excessive alcohol intake (>140 g/week), or extreme physical training (>10

hours/week of intensive exercise), as these factors are known to influence hormone levels.

Blood Sample Collection

Venous blood was collected in lithium heparin-coated tubes between 08:00–10:00 AM after an overnight fast (≥10 hours) and a 30-minute seated rest to establish a standardized, basal hormonal state and minimize stress-induced prolactin elevation.Premenopausal women were sampled during the early follicular phase (days 2–5 of a confirmed regular menstrual cycle) to ensure comparability of baseline sex steroid levels.Postmenopausal status was confirmed by FSH >30 mIU/mL and ≥12 months of amenorrhea.Perimenopausal status was defined for women aged 40–55 years based on the STRAW+10 criteria, characterized by a persistent difference in consecutive cycle length of ≥7 days[6].

Hormone Measurement

Steroid Hormones (Testosterone, Estradiol, Progesterone)

Quantification was performed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).Chromatographic separation was achieved on a C18 column (2.1 × 50 mm, 1.7 µm particle size) with a mobile phase consisting of 0.1% formic acid in water (solvent A) and methanol (solvent B).Electrospray ionization (ESI) in positive mode was employed for ion generation.Calibration utilized isotope-labeled internal standards (testosterone-D3, estradiol-D4, progesterone-D9) to ensure accuracy.The limits of detection (LOD) were <2 pg/mL for estradiol, <0.1 ng/mL for testosterone, and <0.05 ng/mL for progesterone. Intra- and inter-assay coefficients of variation (CVs) were maintained below 10%.In this study, total testosterone was measured using LC-MS/MS. Free or bioavailable testosterone was not calculated, as sex hormone-binding globulin (SHBG) and albumin levels were not measured.

Protein Hormones (LH, FSH, Prolactin)

Measurements were conducted using chemiluminescent immunoassay (CLIA) on a fully automated analyzer (Abbott ARCHITECT).The analytical sensitivity was <0.1 mIU/mL for LH and FSH and <1 ng/mL for prolactin.All samples were analyzed in duplicate, and internal quality control materials were included in each batch to ensure precision and reproducibility.

Statistical Analysis

Data normality was assessed using Shapiro-Wilk tests.Non-normally distributed variables (LH, FSH) underwent log-transformation prior to analysis.Outliers (>3 SD from the mean) were excluded (n=3).Hormone levels are reported as mean ± SD for normally distributed data and median (IQR) for skewed data.Age- and sex-specific reference intervals (2.5th–97.5th percentiles) were derived following IFCC recommendations.Group comparisons used ANOVA with Tukey's post-hoc tests (normally distributed data) or Kruskal-Wallis tests (non-parametric data). Independent t-tests compared sexes.Statistical significance was set at $p<0.05$.Analyses were performed using SPSS v20.0 and R v4.1.2.

Results

Demographic Characteristics

Table 1 presents the baseline characteristics of 500 healthy individuals (250 males, 250 females) aged 19–70 years.The mean age was similar for both sexes (≈ 45 years).Males had a slightly higher BMI (24.3 vs. 23.8 kg/m²) and waist circumference (85.0 vs. 80.5 cm), while females had a larger hip circumference (100.5 vs. 98.0 cm).Waist-to-hip ratio (WHR) was higher in males (0.87 vs. 0.80), reflecting greater abdominal fat accumulation.These differences align with sex-specific fat distribution patterns, which influence age-related hormonal changes analyzed in the study.

Table 1: Demographic and Anthropometric Characteristics of Participants

Variable	Males (n = 250)			Females (n = 250)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Age (years)	44.5 ± 15.2	19	70	45.1 ± 14.8	19	70
BMI (kg/m²)	24.3 ± 3.1	18.5	30.2	23.8 ± 3.0	17.9	29.5
WC (cm)	85.0 ± 8.5	72	102	80.5 ± 7.5	68	95
HC (cm)	98.0 ± 6.2	88	112	100.5 ± 6.8	90	115
WHR	0.87 ± 0.05	0.75	0.98	0.80 ± 0.06	0.7	0.92

Age-Related Hormonal Trends in Males

1.Testosterone levels

Testosterone levels, measured via LC-MS/MS, exhibited a progressive and statistically significant decline with age ($p < 0.001$). The highest levels were observed in the 19--29 age group (6.0 ± 1.2 ng/mL), decreasing linearly to 3.5 ± 0.8 ng/mL in the 60--70 age group **(Table 2, Figure 1A).**

Table 2: Re**presents age-related hormonal changes in males across different age groups.**

Hormone	Age Group (Years)	Mean ± SD	95% CI	p-value
Testosterone(ng/mL)	19–29	6.0 ± 1.2	5.8 – 6.2	—
	30–39	5.2 ± 1.1	5.0 – 5.4	< 0.001
	40–49	3.9 ± 0.9	4.3 – 4.7	< 0.001
	50–59	3.9 ± 0.9	3.7 – 4.1	< 0.001
	60–70	3.5 ± 0.8	3.3 – 3.7	< 0.001
Estradiol(pg/mL)	19–29	35.0 ± 8.0	33.7 – 36.3	—
	30–39	33.5 ± 7.5	32.3 – 34.7	0.23
	40–49	30.0 ± 7.0	29.0 – 31.0	0.004
	50–59	28.0 ± 6.5	27.1 – 28.9	0.0017
	60–70	25.0 ± 6.0	24.1 – 25.9	0.0016
Progesterone(ng/mL)	19–29	0.30 ± 0.08	0.28 – 0.32	—
	30–39	0.28 ± 0.07	0.27 – 0.29	0.08
	40–49	0.25 ± 0.06	0.24 – 0.26	< 0.001

	50–59	0.20 ± 0.05	0.19 – 0.21	< 0.001
	60–70	0.15 ± 0.04	0.14 – 0.16	< 0.001
LH(mIU/mL)	19–29	4.5 ± 1.5	4.3 – 4.7	—
	30–39	5.0 ± 1.6	4.8 – 5.2	< 0.001
	40–49	6.5 ± 2.0	6.2 – 6.8	< 0.001
	50–59	8.0 ± 2.5	7.6 – 8.4	< 0.001
	60–70	9.5 ± 3.0	9.0 – 10.0	< 0.001
FSH(mIU/mL)	19–29	4.0 ± 1.8	3.8 – 4.2	—
	30–39	5.0 ± 2.0	4.7 – 5.3	< 0.001
	40–49	7.5 ± 3.0	7.1 – 7.9	< 0.001
	50–59	12.0 ± 4.5	11.4 – 12.6	< 0.001
	60–70	16.0 ± 5.5	15.3 – 16.7	< 0.001
Prolactin(ng/mL)	19–29	8.5 ± 2.5	8.2 – 8.8	—
	30–39	8.6 ± 2.6	8.3 – 8.9	0.42
	40–49	8.7 ± 2.7	8.4 – 9.0	0.31
	50–59	8.8 ± 2.8	8.5 – 9.1	0.25
	60–70	8.9 ± 2.9	8.5 – 9.3	0.18

2.Estradiol levels

Estradiol remained relatively stable in younger males (35.0 ± 8.0 pg/mL at 19--29 years) but showed a gradual decline in older age groups, reaching 25.0 ± 6.0 pg/mL by 60--70 years **(p = 0.0016; Table 2, Figure 1B).**

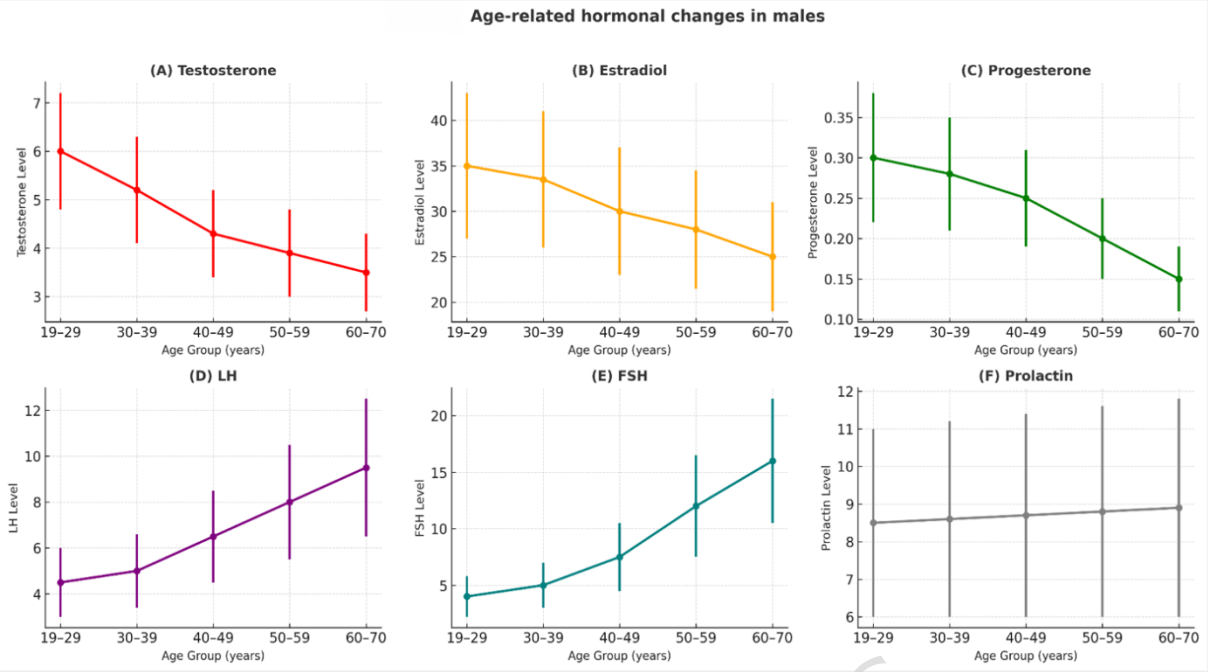

Figure 1 (Males): Age-related changes in hormone levels. (A) Testosterone, (B) Estradiol, and (C) Progesterone show a significant decline with age. (D) Luteinizing Hormone (LH) and (E) Follicle-Stimulating Hormone (FSH) show a significant increase with age. (F) Prolactin levels remain stable across all age groups.

3.Progesterone levels

Progesterone decreased gradually with aging, declining from 0.30 ± 0.08 ng/mL in the youngest group to 0.15 ± 0.04 ng/mL in the oldest group **($p < 0.001$; Table 2, Figure 1C).**

4.Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH)

LH and FSH levels rose progressively with age ($p < 0.001$). LH increased from 4.5 ± 1.5 mIU/mL (19--29 years) to 9.5 ± 3.0 mIU/mL (60--70 years), while FSH surged from 4.0 ± 1.8 mIU/mL to 16.0 ± 5.5 mIU/mL in the same period (Table 2, Figure 1D--E).

5.Prolactin Levels

Prolactin remained stable across all male age groups (pooled mean: 8.7 ± 2.7 ng/mL; p = NS; **Table 2**, **Figure 1F**).

Age-Related Hormonal Trends in Females

Female participants were categorized by reproductive status (premenopausal: 19–45 years; perimenopausal: 45–50 years; postmenopausal: ≥50 years).

1.Testosterone Levels

Testosterone levels were significantly lower in females than males ($p < 0.001$). Premenopausal women exhibited mean levels of 0.40 ± 0.15 ng/mL, declining to 0.18 ± 0.07 ng/mL postmenopause **($p < 0.001$; Table 3, Figure 2A).**

Table 3: **summarizes hormonal variations in females before and after menopause.**

Hormone	Age Group (Years)	Reproductive Status	Mean ± SD	95% CI	p-value
Testosterone(ng/mL)	19–29	Premenopausal	0.40 ± 0.15	0.37 – 0.43	—
	30–39	Premenopausal	0.35 ± 0.12	0.33 – 0.37	0.12
	40–49	Perimenopausal	0.28 ± 0.10	0.26 – 0.30	< 0.001
	50–59	Postmenopausal	0.20 ± 0.08	0.19 – 0.21	< 0.001
	60–70	Postmenopausal	0.18 ± 0.07	0.17 – 0.19	< 0.001
Estradiol(pg/mL)	19–29	Premenopausal	110.0 ± 50.0	102.8 – 117.2	—
	30–39	Premenopausal	100.0 ± 45.0	93.4 – 106.6	0.15
	40–49	Perimenopausal	65.0 ± 30.0	60.7 – 69.3	< 0.001
	50–59	Postmenopausal	25.0 ± 10.0	23.7 – 26.3	< 0.001

	60–70	Postmenopausal	20.0 ± 8.0	19.0 – 21.0	< 0.001
Progesterone(ng/mL)	19–29	Premenopausal	3.5 ± 2.5*	3.1 – 3.9	—
	30–39	Premenopausal	3.2 ± 2.2*	2.9 – 3.5	0.09
	40–49	Perimenopausal	0.8 ± 0.6	0.7 – 0.9	< 0.001
	50–59	Postmenopausal	0.3 ± 0.2	0.28 – 0.32	< 0.001
	60–70	Postmenopausal	0.2 ± 0.1	0.19 – 0.21	< 0.001
LH(mIU/mL)	19–29	Premenopausal	6.5 ± 3.0	6.1 – 6.9	—
	30–39	Premenopausal	7.0 ± 3.5	6.5 – 7.5	0.45
	40–49	Perimenopausal	18.0 ± 8.0	16.9 – 19.1	< 0.001
	50–59	Postmenopausal	30.0 ± 12.0	28.5 – 31.5	< 0.001
	60–70	Postmenopausal	32.0 ± 13.0	30.4 – 33.6	< 0.001
FSH(mIU/mL)	19–29	Premenopausal	7.0 ± 3.5	6.5 – 7.5	—
	30–39	Premenopausal	7.5 ± 4.0	6.9 – 8.1	0.1
	40–49	Perimenopausal	25.0 ± 10.0	23.7 – 26.3	< 0.001
	50–59	Postmenopausal	65.0 ± 20.0	62.5 – 67.5	< 0.001
	60–70	Postmenopausal	70.0 ± 22.0	67.3 – 72.7	< 0.001
Prolactin(ng/mL)	19–29	Premenopausal	12.5 ± 4.5	11.9 – 13.1	—
	30–39	Premenopausal	12.6 ± 4.6	12.0 – 13.2	0.68
	40–49	Perimenopausal	12.23 ± 1.38	12.1 – 13.3	0.52
	50–59	Postmenopausal	12.35 ± 1.42	12.2 – 13.4	0.37
	60–70	Postmenopausal	12.42 ± 1.45	12.3 – 13.5	0.29

*Note: The high SD for progesterone in premenopausal women reflects the large variation between the follicular (low) and luteal (high) phases of the menstrual cycle. Samples were taken in the early follicular phase, but some variability remains.

2.Estradiol Levels

Premenopausal estradiol levels (110.0 ± 50.0 pg/mL) dropped sharply postmenopause (20.0 ± 8.0 pg/mL; $p < 0.001$), with the steepest decline occurring during perimenopause (65.0 ± 30.0 pg/mL; **Table 3, Figure 2B).**

3.Progesterone Levels

Progesterone decreased markedly after menopause (premenopausal follicular phase: 3.5 ± 2.5 ng/mL; postmenopausal: 0.2 ± 0.1 ng/mL; $p < 0.001$), reflecting ovarian senescence **(Table 3, Figure 2C).**

4.Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH)

LH and FSH surged postmenopause (LH: 32.0 ± 13.0 mIU/mL; FSH: 70.0 ± 22.0 mIU/mL), consistent with the loss of ovarian feedback inhibition **($p < 0.001$; Table 3, Figure 2D--E).**

5.Prolactin Levels

Prolactin levels remained stable across all female age groups (pooled mean: 12.7 ± 4.7 ng/mL; $p > 0.05$; **Table 3, Figure 2F**).

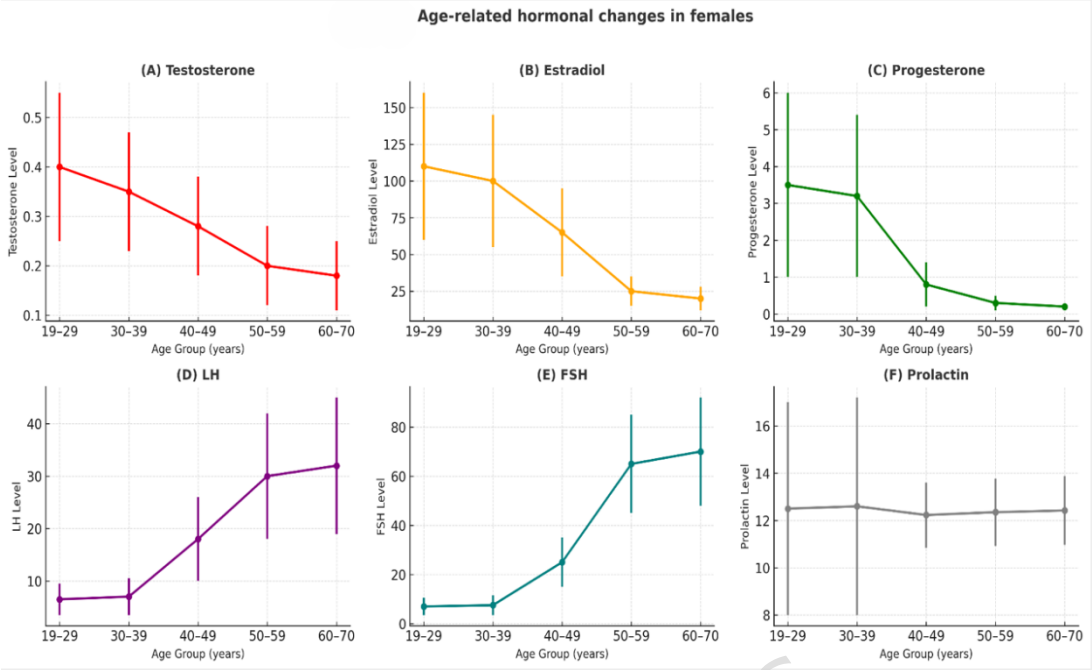

Figure 2 (Females): Age-related changes in hormone levels. (A) Testosterone, (B) Estradiol, and (C) Progesterone show a significant decline, particularly post-menopause. (D) Luteinizing Hormone (LH) and (E) Follicle-Stimulating Hormone (FSH) show a significant increase post-menopause. (F) Prolactin levels remain stable across all age groups.

Discussion

This study provides detailed age- and sex-specific reference intervals for key reproductive hormones in a healthy Chinese population, addressing a critical gap in Asian-specific endocrine data.Our findings delineate clear hormonal trajectories with aging, highlighting patterns that both align with and diverge from established literature.The use of LC-MS/MS for steroid hormone analysis in this context contributes valuable methodological

precision to the field.A central finding concerns the age-related trajectory of testosterone in men.We observed a progressive, statistically significant decline in total testosterone with advancing age.This finding contrasts with several large Chinese cohorts that utilized immunoassays and reported stable or even rising testosterone levels with age [3, 4, 7-9].This discrepancy likely reflects key methodological and population differences.The use of gold-standard LC-MS/MS in our study minimizes cross-reactivity issues inherent in steroid immunoassays, potentially providing a more accurate quantification[5, 6]. Furthermore, our strict exclusion criteria for comorbidities and lifestyle factors may have isolated a more pronounced physiological aging effect, distinct from the influences of prevalent age-related conditions that can confound trends in larger, less stringently defined community cohorts.This underscores the importance of defining "health" and measurement methodology when establishing and applying reference intervals.

In females, our data captured the expected dramatic endocrine shifts of the menopausal transition.The sharp postmenopausal declines in estradiol and progesterone, coupled with the compensatory surge in gonadotropins (LH and FSH), are classic hallmarks of ovarian senescence [1, 6]. The stability of prolactin across all age groups in both sexes reinforces its value as a reliable biomarker, where deviations are more indicative of underlying pathology (e.g., prolactinomas) than normal aging processes[10].

The observed gradual decline in progesterone in aging males is a novel descriptive finding; its clinical significance remains unclear and warrants further investigation.The primary clinical utility of this study lies in providing population-specific, age-adjusted reference intervals.These benchmarks will aid clinicians in distinguishing between normal, age-related hormonal changes and pathological deficiencies. For instance, a 65-year-old man with a testosterone level at the lower end of the reference range for his age in this healthy cohort may be within the expected physiological range, potentially avoiding unnecessary treatment, whereas the same level in a 30-year-old would warrant investigation. Similarly, understanding the expected gonadotropin surge in postmenopausal women prevents misinterpretation of elevated LH and FSH as abnormal.

Limitations

Several limitations of this study should be considered when interpreting the results.First, the cross-sectional design provides a population-level snapshot of hormonal aging but cannot track intra-individual trajectories over time; longitudinal studies are needed for this purpose.Second, the lack of SHBG measurement precludes the assessment of free or bioavailable testosterone.While some consider this the biologically active fraction, it is a calculated rather than a directly measured analyte, which introduces its own limitations.Third, this single-center recruitment strategy from a blood donor population may introduce a "healthy donor effect," as these individuals are typically free of acute illness, have normal hemoglobin levels, and may overall be healthier than the general population.This may limit the generalizability of our findings to the broader

Chinese population, which includes individuals with unmeasured subclinical conditions or different health-seeking behaviors.Furthermore, while we excluded individuals based on extreme behaviors, we did not quantitatively adjust for potential confounders such as diet, stress, or moderate exercise levels. Finally, although our health screening was rigorous, the absence of direct verification of normal reproductive function (e.g., via semen analysis in men) remains a constraint for a study establishing reproductive hormone references [5].

Conclusion

This study establishes comprehensive, age- and sex-specific reference intervals for key reproductive hormones in a healthy Chinese cohort, addressing a significant gap in population-specific endocrine data.By utilizing the gold-standard LC-MS/MS methodology for steroid hormones and implementing stringent selection criteria, this work provides a robust foundation for distinguishing normative, age-related hormonal changes from potential pathological states within this demographic.These findings underscore the critical importance of applying population- and methodology-appropriate benchmarks in clinical practice to enhance diagnostic accuracy and guide appropriate management, such as avoiding unnecessary hormonal treatments in cases of physiological decline.The novel observation of a gradual progesterone decline in aging males highlights an area for further mechanistic exploration.Future longitudinal studies are needed to validate these reference intervals, track intra-individual hormonal trajectories, and further refine personalized assessment frameworks for endocrine health across the lifespan.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author Contribution

Y.A.: administrative support, provision of study materials or subjects, and manuscript writing. Y.A.: collection and assembly of data, and manuscript writing. Y.A.; S.B.A.: provision of study materials or subjects, data analysis, and interpretation.; S.B.A.; R.T.D.; S.B.; M.H.; J.C.; L.C.W.; W.S.: collection and assembly of data, data analysis and interpretation, and manuscript writing. W.S.: conception and design, and final approval of the manuscript. All authors have read and approved the manuscript.

Ethics statement

The need for formal ethics approval was waived by the Institutional Ethics Committee of the affiliated hospital of Yangzhou university, as the study involved collection of anonymized leftover blood samples from the Department of Blood Transfusion. The samples were obtained with institutional permission. This procedure is in accordance with the Declaration of Helsinki and complies with national guidelines regarding the use of secondary clinical material for research.

Informed Consent Statement

Informed consent was obtained from all participants before their inclusion in the study. Due to privacy and confidentiality considerations, copies of the signed consent forms are not publicly available

Data Availability

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Clinical trial number: Since this is a cross-sectional study and not a clinical trial, we state:Clinical trial number: not applicable.

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