International Journal of Health Sciences and Research
[Indexed, Peer-Reviewed Journal]
Volume 15; Issue: 9; September 2025
Website: www.ijhsr.org
Review Article *ISSN: 2249-9571*

Diagnostic Challenges Associated with Rhythmic, Pulsatile Hormones in Humans

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DOI: https://doi.org/10.52403/ijhsr.20250954

ABSTRACT

Pulsatile hormones are essential for regulating growth, metabolism, and reproduction in humans. These hormones are released in bursts, with levels fluctuating based on circadian rhythms and physiological demands. For example, growth hormone (GH) is secreted during sleep to promote tissue repair, while luteinizing hormone (LH) pulses drive ovulation and fertility. This review shows recent advances in understanding pulsatile hormones and their circadian rhythms, and for the first time, systematically outlines the diagnostic challenges posed by their pulsatile nature. We also propose mitigation strategies for pre-analytical variability and suggest alternative tests to improve diagnostic accuracy.

Keywords: Pulsatile hormones; circadian rhythms; follicle-stimulating hormone; luteinizing hormone; prolactin; growth hormone; thyroid-stimulating hormone; insulin; adrenocorticotropic hormone; cortisol

1. INTRODUCTION

Pulsatile hormones in the human body and their physiological roles. They regulate numerous physiological functions, including growth, metabolism, and reproduction (1). These hormones are secreted in bursts or pulses throughout the day, with levels fluctuating based on the time of day and particular physiological requirements (2). The pulsatile characteristics of these hormones facilitate exact regulation of physiological activities and reactions to fluctuating internal and external situations. For instance, growth hormone (GH) is secreted in pulses during sleep to facilitate tissue repair and growth (3). The pulsatile release of reproductive hormones, such as luteinizing hormone (LH), is crucial for ovulation and female fertility (4). The pulsatile secretion of hormones enables the body to respond effectively to diverse stimuli and sustain homeostasis. This complex system of hormone regulation is essential for general health and wellness. The present review highlights gaps in current knowledge, summarizes recent research, and addresses diagnostic challenges unique to pulsatile hormones.

2. PULSATILE HORMONE SECRETION AND PHYSIOLOGICAL IMPACT

Pulsatile hormones, secreted in bursts or pulses, play a crucial role in physiological functions. Key pulsatile hormones include LH and follicle-stimulating hormone (FSH), which regulate reproductive functions; growth hormone, which influences growth, cell repair, and metabolism (5)(6); thyroid-stimulating hormone (TSH), which

stimulates thyroid hormone secretion (7); insulin, which manages blood glucose levels (8); adrenocorticotropic hormone (ACTH) and cortisol, which regulate metabolism, immune response, and stress (9); and prolactin, which facilitates milk production by mammals (10). These hormones work together to regulate various bodily functions.

Pulsatile secretion ensures that target tissues remain sensitive to these hormones, optimizing their effects. It's fascinating how our bodies use these rhythms to maintain balance and health. The body's hormones regulate reproductive functions, growth, cell repair, metabolism, insulin, cortisol, and thyroid hormones. They also regulate blood glucose levels, immune response, stress, and maintain calcium and phosphate levels (11).

Overall, hormones play a crucial role in maintaining homeostasis within the body by ensuring that various bodily functions are properly regulated. Any disruption in hormone levels can lead to significant health issues and imbalances throughout the body (12). For example, imbalances in insulin levels can lead to diabetes, while disruptions in thyroid hormone levels can result in conditions like hypothyroidism or hyperthyroidism. It is essential to monitor hormone levels and seek medical attention if any abnormalities are detected to prevent potential health complications (13). GH exhibit high spikes during sleep, FSH exhibit regular pulses, LH exhibit regular pulses, thyroid-stimulating hormones exhibit slight diurnal variation, and insulin spikes occur post-meal. Pulsatile secretion is characterized by spikes or peaks at regular intervals (**Figure 1, Table 1**).

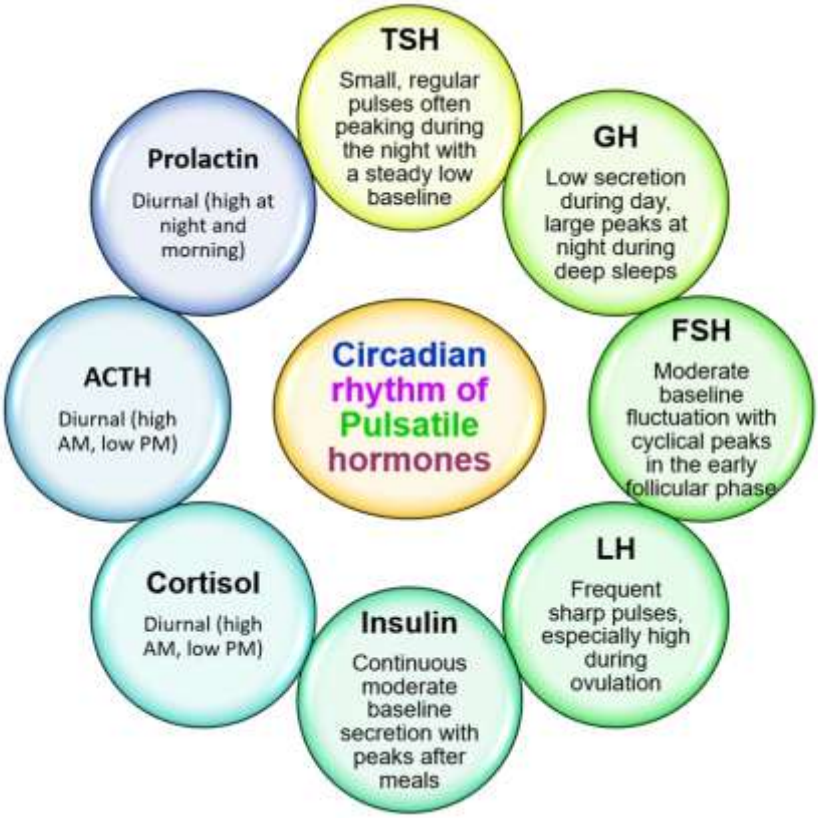

Figure 1: Challenges associated/ circadian rhythm of pulsatile hormones in human

Table 1. The tables emphasize the primary characteristics of pulsatile hormone secretion, their circadian rhythms, functions, and the associated conditions.

Name of the Hormone	Pulsatile secretary pattern	Circadian rhythm	Physiological function	Associated conditions
FSH	Ultradian bursts	Menstrual cycle/ physiological factors, rather than a 24-hour cycle	Reproduction	High levels: menopause, primary ovarian insufficiency, or pituitary disorders. Low levels: hypogonadism, pituitary dysfunction, or reproductive issues (14).
LH	Ultradian (pulses)	Diurnal (peaks in morning)	Reproduction	Polycystic ovary syndrome (PCOS) (15).
Prolactin	Ultradian (95 min pulses	Diurnal (high at night and morning)	Lactation, reproduction, immunity, metabolism	Hyperprolactinemia, hypoprolactinemia, prolactin-secreting pituitary adenomas (16).
GH	Ultradian (pulses)	Diurnal (high at night)	Growth, metabolism	Acromegaly, GH deficiency (17)
TSH	Ultradian (pulses)	Diurnal (peaks in night)	Thyroid function	Hyperthyroidism, hypothyroidism (18)
Insulin	Ultradian (pulses)	Diurnal (peaks post-meal)	Glucose metabolism	Diabetes mellitus (8)
ACTH	Ultradian (pulses)	Diurnal (high AM, low PM)	Produce and release cortisol, Production of androgens	Cushing's syndrome, Addison's disease (19)
Cortisol	Ultradian (90 min pulses)	Diurnal (high AM, low PM)	Stress response, metabolism	Cushing's syndrome, Addison's disease (19)

2.1. Regulation of hormones

2.1.1. Hypothalamic-pituitary axis

The hypothalamic-pituitary axis (HPA) is essential for the regulation of hormone secretion in the body. Homeostasis is sustained through a multifaceted interaction between the hypothalamus and the pituitary gland (20).

Hypothalamus: Located beneath the thalamus in the brain. Serves as the primary regulatory center of the body, regulating a variety of autonomic functions, including temperature, appetite, thirst, and circadian rhythms (21).

Pituitary Gland: An organ that is approximately the size of a pea, situated at the base of the brain. Referred to as the "master gland," it secretes hormones that regulate the activity of other endocrine glands in the body (22).

2.1.2. Hypothalamic regulation

The hormones released by the hypothalamus suppress hormones like somatostatin and dopamine while stimulating or inhibiting the secretion of pituitary hormones like corticotropin-releasing hormone (CRH), gonadotropin-releasing hormone (GnRH), and thyrotropin-releasing hormone (TRH). Numerous body processes, such as metabolism, stress response, and reproductive function, are regulated by these hormones. Maintaining general health and homeostasis in the body depends on the complex balance of these hormones (23).

2.1.3. Secretion of Pituitary Hormones

Two main parts make up the pituitary gland, which controls growth, metabolism, and reproduction by secreting hormones such as GH, ACTH, TSH, prolactin, FSH, and LH. The pituitary gland, which is located at the base of the brain, is frequently called the "master gland" because of its important function in regulating the body's hormone levels. Medical intervention may be necessary for the appropriate management of a variety of health issues caused by pituitary gland dysfunction (24).

The hormones oxytocin and vasopressin are secreted by the posterior pituitary. These hormones are produced in the hypothalamus and are responsible for regulating uterine contractions, milk ejection, and hydration balance. Birth, nursing, and blood pressure regulation are all greatly impacted by these hormones. During labor or when the body needs to conserve water, they are released in response to signals from the hypothalamus (25).

2.1.4. Mechanisms for feedback

There are positive and negative feedback loops that the hypothalamus and pituitary (HPA) use to control hormone levels. When blood levels rise above a certain point, the production and release of stimulating hormones are inhibited by negative feedback. On the other hand, processes such as the luteal surge occasionally involve positive feedback. Disruptions in the HPA axis can result in a variety of endocrine disorders; this axis is essential for the regulation of stress, growth, reproduction, the internal environment of the body, and metabolism (23). The effects on general health and wellness of disorders like Cushing's syndrome and Addison's disease, which arise from disturbances in the HPA axis, are substantial. Healthcare providers must have a deep understanding of the HPA axis to properly diagnose and treat endocrine disorders (26).

3. DIAGNOSTIC CHALLENGES

Hormonal measurement is a valuable tool in the diagnosis of a variety of endocrine disorders:

3.1. FSH and LH: FSH and LH are essential for the regulation of the reproductive system, and detecting disorders related to these hormones can be intricate. Below are certain diagnostic problems frequently encountered.

Hypogonadism is a condition where hormone levels are measured based on various factors such as age, gender, and the menstrual cycle or reproductive stage. Pulsatile secretion of FSH and LH by the pituitary gland makes isolated measurements less accurate. Deviant levels can indicate primary gonadal failure, while reduced levels may indicate secondary hypogonadism due to hypothalamic or pituitary dysfunction. Symptomatic overlap can complicate diagnosis, as disorders like PCOS, hypothyroidism, or hyperprolactinemia may present with similar symptoms (27). External factors like medications, stress, and systemic disorders can also cause misunderstandings. Laboratory standardization and non-specific symptoms, such as fatigue, depression, and decreased libido, can complicate diagnosis (28). Testosterone levels fluctuate throughout the day and can be affected by factors like stress, illness, and medications (29). Distinguishing between age-related declines in testosterone and true hypogonadism can be challenging, as older men often have lower levels without clinical symptoms. Assay variability and underlying causes of secondary hypogonadism require additional testing and expertise (30). Patient awareness of hypogonadism symptoms and the importance of seeking medical evaluation can also contribute to underdiagnosis.

3.2. Prolactin: Diagnosing prolactin-related disorders is complex due to various factors such as physiological variations, medications, pituitary disorders, systemic conditions, clinical interpretation, and stress effects. Prolactin levels naturally vary based on factors such as time of day, pregnancy, breastfeeding, stress, exercise, and certain meals (31). High levels of macroprolactin, a biologically inactive form of prolactin, can lead to falsely elevated test results (32). An extensive medication history is necessary for the identification of falsely elevations. An increase in prolactin levels can occur as a result of a disruption in dopamine inhibition in pituitary illnesses such prolactinomas. Hypothyroidism, polycystic ovary syndrome, and chronic renal disease are among systemic illnesses that can

increase prolactin levels (33). Clinical interpretation can be challenging as symptoms of elevated prolactin can overlap with other hormonal or systemic issues. Stress effects during blood collection can also transiently elevate prolactin levels, potentially leading to false-positive results. To address these challenges, clinicians typically use repeated prolactin measurements, dynamic testing, imaging studies, and a thorough review of the patient's medical, medication, and lifestyle history.

3.3. Growth hormone deficiency:
Clinicians can differentiate between normal fluctuations and pathological inadequacies by observing the pulsatile production of growth hormone (GH). The evaluation of pulsatility is crucial in averting the risk of misdiagnosis that could arise from a solitary random test (34).
Diagnosing GH deficiency presents several challenges:
Diagnosing growth hormone insufficiency is challenging due to non-specific symptoms, including reduced growth rate in children and diminished muscle mass and energy in adults, which may overlap with other illnesses (35). The diagnosis is further complicated by the manifestation of GH deficit, which might present as increased adiposity, reduced bone density, and elevated cardiovascular risk factors. Consequently, a thorough assessment involving blood testing and imaging scans may be essential for the precise diagnosis of GH insufficiency (34).
Growth hormone levels vary throughout the day, rendering individual measurements inaccurate. Sample collection at 8 am is deemed appropriate due to circadian rhythms (36). Growth hormone stimulation tests are intricate and protracted, with potential risks of undesirable effects. Variables such as age, sex, and BMI can influence GH response, complicating the diagnostic process. Insufficient awareness may result in postponed diagnosis and adversely affect treatment outcomes (37). Healthcare practitioners must consider these aspects while evaluating GH test findings and formulating suitable treatment options. Patients must be informed about the significance of prompt diagnosis and intervention to enhance outcomes and quality of life.
Transition phase: The period from late puberty to early adulthood is critical for reassessing GH levels and deciding on continued therapy, but evidence-based guidelines for this phase are lacking.

3.4. Thyroid stimulating hormone:
Macro-TSH is an uncommon syndrome in which TSH associates with other plasma proteins, typically immunoglobulins, resulting in inaccurately raised TSH levels (38). This interference may result in the misdiagnosis of thyroid diseases and unwarranted treatment. Healthcare providers must recognize these phenomena and contemplate alternative diagnostic approaches in suspected instances of Macro-TSH. This may simulate subclinical hypothyroidism and result in improper treatment. Interference in immunoassays, including heterophilic antibodies, can compromise the precision of TSH immunoassays (39). Fluctuations in TSH levels, affected by stress, sickness, and pharmacological agents, render individual assessments less dependable. The influence of age and BMI may obfuscate the interpretation of results (40). Analytical mistakes may lead to inconsistencies between biochemical test outcomes and clinical observations. Consequently, it is essential to account for potential interferences and confounding variables while analyzing TSH levels. Furthermore, repeated testing and clinical correlation may be essential for the precise diagnosis of thyroid dysfunction.

3.5. Insulin:
Insulin-related disorders can be challenging to detect because of symptom overlap, variable insulin levels, intricate diagnostic procedures such as the 72-hour fasting test,

assay interference, and the difficulty in identifying tumors (41). Symptoms associated with insulin-related disorders may encompass confusion, dizziness, and syncope, potentially leading to misdiagnosis of alternative ailments (41,42). Elements such as stress, nutrition, and pharmacological treatments can also affect insulin levels, complicating the diagnostic process. The definitive method for diagnosing insulinoma is the 72-hour fasting test, which is labor-intensive and necessitates meticulous observation (43). Interference in assays might result in erroneous outcomes, complicating the identification of the precise source of insulin-related symptoms. Imaging studies may be required to identify tumors responsible for aberrant insulin levels in certain instances. Moreover, variations in insulin sensitivity and metabolism among individuals can influence test outcomes, requiring tailored interpretation (44). Healthcare providers must examine all these criteria while assessing a patient for insulinoma. Collaboration among endocrinologists, radiologists, and other specialists may be essential for the precise diagnosis and treatment of this rare illness.

3.6. ACTH and Cortisol: Variability in Test Results: The results of various assays and testing methodologies may differ. For instance, the Siemens Immulite ACTH assay and the Roche Elecsys ACTH assay can generate ACTH levels that differ for the same patient (45).

The collection of samples is essential for ensuring consistency in ACTH levels, which may vary throughout the day. The morning collection guarantees consistency. Proper sample handling and processing is crucial, requiring plasma to be frozen within 15 minutes and blood samples to be taken in pre-chilled tubes and immersed in cold water. This will aid in averting sample degradation and guarantee precise outcomes. Adhering to precise protocols for sample collection is crucial to reduce variability in ACTH levels (46).

Results Interpretation: The interpretation of ACTH levels necessitates a meticulous examination of the patient's clinical profile and other diagnostic tests. For example, ACTH levels can assist in the distinguishing between primary adrenal insufficiency (Addison's disease) and secondary adrenal insufficiency.

ACTH levels within the "gray zone" (5-20 pg/mL) can be less definitive and require additional testing and clinical correlation. Diagnosing ACTH-related disorders requires a comprehensive approach, precision, and accuracy (47). The secretion of cortisol is characterized by a unique circadian and ultradian rhythm, making it difficult to distinguish between Cushing's syndrome and pseudo-Cushing's states. Non-specific symptoms like weight gain, fatigue, and hypertension can make it difficult to identify without specific testing (48). Fluctuating cortisol levels can be affected by stress, medications, and other factors, complicating the diagnosis. Cyclic Cushing's syndrome involves periods of high cortisol levels interspersed with normal levels, making it harder to detect consistently. Identifying excess cortisol from adrenal tumors or ectopic sources can be challenging (49). Incidental adrenal masses (incidentalomas) during imaging for other conditions can lead to overdiagnosis if not properly evaluated. Diagnostic tests like the dexamethasone suppression test, 24-hour urinary free cortisol, and late-night salivary cortisol have limitations and can sometimes yield false positives or negatives (47).

FSH and LH tests are recommended for women on day 2 or 3 of the menstrual cycle (50). GH tests are conducted on morning samples, with options including IGF-1, GH stimulation/suppression tests, TSH tests, insulin tests, and ACTH tests (42,45,51). Insulin tests are conducted fasting, with options including fasting glucose, HbA1c, fructosamine, and C-peptide tests (52). ACTH tests are conducted at 7 AM and 10 AM due to diurnal variation. Cortisol tests are conducted at 7-10 AM, with options

including salivary and urinary cortisol tests (**Table 2**).

Table 2. Sample collection timed, container and alternative tests for pulsatile hormones

Test name	Sample collection timed	Preferred container	Transportation
FSH and LH	Women- on day 2 or 3 of the menstrual cycle	Gold top tube	2-8°C
Prolactin	To be collected exactly 2 hours after waking up to ensure reliable results	Gold top tube (SST)	2-8°C (stable for 48 hours)
GH	Morning sample	Serum gel tube or a red top tube or aprotinin tube*	2-8°C (stable for 48 hours)
TSH	Morning sample	Gold top (serum separator-SST gel) tube or Mint green top (lithium heparin gel) tube	2-8°C (stable for 48 hours)
Insulin	Recommended to fast for at least 8 hours before the test	Gold top tube (SST) or a red top tube (clot activator)	2-8°C
ACTH	7 AM and 10 AM due to the diurnal variation of ACTH levels	Lavender-top (EDTA) tube	2-8°C (stable for 48 hours)
Cortisol	7-10 am	Red-top/ gold-top tube	2-8°C (stable for 48 hours)
*NOTE- Aprotinin tubes are specialized blood collection vessels used in research and diagnostic applications for testing proteolytic enzyme inhibitors, consisting of K3EDTA as an anticoagulant and Aprotinin for protein preservation.			

The subsequent assays are employed to assess many hormones, including FSH, LH, GH, TSH, insulin, ACTH, cortisol, and prolactin. FSH and LH assess ovarian reserve, estrogen concentrations, and endometrial receptivity (53). GH tests monitor the hormonal activity and secretion, identify GH insufficiency, and assess diseases such as acromegaly. TSH tests are utilized to diagnose and monitor thyroid abnormalities as well as to identify autoimmune thyroid illnesses (54). Insulin tests diagnose and monitor diabetes, evaluate glucose regulation, and distinguish between kinds. ACTH tests assess pituitary and adrenal functionality and detect adrenal insufficiency and related diseases. Cortisol testing evaluate adrenal function and identify diseases related to cortisol production (49). Macroprolactin assays distinguish between macroprolactinemia and genuine hyperprolactinemia for precise diagnosis and management (38). Endocrine testing is essential for detecting and controlling several hormonal diseases. These assessments assist healthcare professionals in making educated judgments about treatment strategies and evaluating the efficacy of interventions over time **(Table 3)**.

Table 3. Suggested alternative approaches to address the diagnostic challenges

Test name	Alternatives tests	Justification
FSH and LH	AMH, E2, progesterone	AMH (Anti-Müllerian Hormone): Indicates the quantity and quality of eggs that are left, serving as an indicator of ovarian reserve. It is a consistent marker that can be assessed at any point during the menstrual cycle. E2 (Estradiol): Determines the concentration of estrogen, which is essential for the development of the endometrium and follicles. The endometrium attains the requisite thickness for successful implantation when the levels are sufficient. Progesterone: Evaluates the hormone that is responsible for endometrial receptivity, a critical factor in the maintenance of pregnancy. It is typically assessed during the luteal phase of the menstrual cycle.
GH	Insulin-like growth factor 1 (IGF-1) test, IGF-BP3, GH stimulation test, growth hormone suppression test	IGF-1 (Insulin-Like Growth Factor 1) Test: The average amount of GH produced by the pituitary gland is represented by IGF-1 levels. This test offers a dependable estimate of GH activity, as IGF-1 levels remain consistent throughout the day, in contrast to GH, which fluctuates. IGF-BP3 (Insulin-Like Growth Factor Binding Protein 3) is the primary carrier protein for IGF-1 in the circulation and is a reflection of GH secretion. It is especially beneficial in the diagnosis of GH deficiency in adolescents. GH stimulation test: This test assesses the pituitary gland's ability to release GH in response to particular stimuli. When GH levels are abnormally low, it aids in the diagnosis of GH deficiency. GH suppression test: This examination assesses the body's capacity to inhibit the production of GH. It is especially beneficial for the diagnosis of acromegaly, a condition in which the body generates an excessive amount of GH.
TSH	Free T4, free T3, Anti-TPO/TG antibodies	Free T4 (Thyroxine): Indicates the quantity of free thyroxine in the bloodstream, which is essential for energy production and metabolism. It is beneficial to evaluate thyroid function and diagnose conditions such as hypothyroidism or hyperthyroidism. Free T3 (Triiodothyronine): Assesses the concentration of free triiodothyronine in the bloodstream. It is particularly beneficial for the diagnosis of hyperthyroidism and the surveillance of the treatment of thyroid disorders. Anti-TPO (Thyroid Peroxidase) Antibodies: Tests for the presence of antibodies against thyroid peroxidase, an enzyme that is essential for the production of thyroid hormones. It aids in the diagnosis of autoimmune thyroid diseases, such as Hashimoto's thyroiditis. Anti-TG (Thyroglobulin) Antibodies: Detects antibodies against thyroglobulin, a protein required for the synthesis of thyroid hormones. This test is beneficial for the diagnosis of autoimmune thyroid disorders and the monitoring of thyroid cancer treatment.
Insulin	Fasting glucose, Hemoglobin A1c (HbA1c), Fructosamine, C-peptide test	Fasting Glucose: This test measures the blood sugar level after an overnight fast, which aids in the diagnosis of diabetes and the evaluation of overall glucose metabolism. Hemoglobin A1c (HbA1c): This test is used to diagnose and monitor diabetes, as well as to evaluate long-term glucose control. Fructosamine: This test is useful in situations where HbA1c may not be reliable, such as in patients with hemoglobinopathies.

		C-Peptide Test: This test measures the level of C-peptide, which is released in equal amounts to insulin. It is used to differentiate between type 1 and type 2 diabetes and to evaluate endogenous insulin production.
ACTH	Metyrapone stimulation test, dehydroepiandrosterone sulphate test	Metyrapone Stimulation Test: This test evaluates the pituitary gland's capacity to produce ACTH in response to metyrapone, a drug that suppresses cortisol production. It is beneficial for the diagnosis of adrenal insufficiency and the assessment of pituitary function. Dehydroepiandrosterone Sulphate (DHEA-S) Test: Determines the concentration of DHEA-S, an adrenal androgen. It is beneficial in the assessment of adrenal function and can assist in the diagnosis of adrenal insufficiency, adrenal tumors, and reproductive development disorders.
Cortisol	Salivary cortisol/ Urinary cortisol	Salivary Cortisol: Evaluates the diurnal variation of cortisol production by measuring the level of cortisol in the saliva, which is frequently collected at various stages of the day. This examination is convenient and non-invasive, rendering it appropriate for the diagnosis of conditions such as Cushing's syndrome and the evaluation of adrenal function. Urinary Cortisol: Quantifies the quantity of cortisol that is excreted in the urine over a 24-hour period. This test is beneficial for the diagnosis of cortisol production disorders, including Cushing's syndrome and Addison's disease, and it assists in evaluating the total daily cortisol production.
Prolactin	Macroprolactin (polyethylin glycol precipitation test)	Macroprolactin (Polyethylene glycol precipitation Test): Quantifies the concentration of macroprolactin, a biologically inactive form of prolactin with a high molecular weight. This test is employed to distinguish between macroprolactinemia and true hyperprolactinemia, thereby guaranteeing an accurate diagnosis and the most suitable treatment.

DISCUSSION

Pulsatile hormones are released in short bursts or pulses by the endocrine glands, rather than being steadily secreted. This pulsatile release is important for maintaining the body's internal balance and responding to changing conditions. Additionally, disruptions in the pulsatile pattern of hormone release can lead to various health issues such as infertility or metabolic disorders (55).

Polycystic Ovary Syndrome (PCOS) is a condition characterized by the abnormal secretion of LH in comparison to FSH, which can be difficult to diagnose without thorough testing. The irregular pattern in question may serve as a critical diagnostic indicator. Non-specific symptoms, such as irregular periods, acne, and weight gain, can be common in other conditions, making it difficult to diagnose PCOS without thorough testing. There are multiple diagnostic criteria, and many women experience significant delays in diagnosis, often waiting years before receiving a proper diagnosis (56).

Diagnosis and management often require a multidisciplinary approach, which can be challenging to coordinate. LH and FSH levels can fluctuate throughout the menstrual cycle, making it difficult to interpret single measurements without considering the timing of the test. Age and menopausal status significantly affect LH and FSH levels, requiring age-specific reference ranges for accurate interpretation. Symptoms of hormonal imbalances, such as irregular periods or infertility, can overlap with other conditions, necessitating comprehensive evaluations (57). Different laboratories may use varying methods to measure LH and FSH, leading to inconsistent results.

Functional Hypothalamic Amenorrhea (FHA) is another condition that can be challenging to distinguish from PCOS due to high interindividual variability in LH and FSH levels (58). GH is secreted in a pulsatile manner, with maximum pulses occurring every 3-5 hours during sleep, exercise, and fasting. Its physiological functions include promoting growth, protein synthesis, and lipolysis. Diagnostic assessment of GH disorders can present as either an excess or a deficiency, and the pulsatile nature of GH secretion necessitates a meticulous evaluation (3).

TSH is a hormone secreted by the anterior pituitary gland in a pulsatile manner, with surges occurring every 2-4 hours (59). TSH plays a primary role in regulating the metabolic process, development and expansion, body temperature, cardiac output, and heart rate. Diagnosis of TSH can be challenging due to its fluctuating levels throughout the day, assay variability, non-thyroidal illness (NTI), and medication effects on TSH secretion. TSH measurement is the initial test used to evaluate thyroid function, with elevated TSH levels indicative of hypothyroidism and reduced TSH levels indicating hyperthyroidism.

Insulin is a crucial component of glucose homeostasis, secreted by beta cells of the pancreas in a pulsatile manner. Pulsatile insulin secretion is more effective than continuous secretion in reducing insulin resistance and improving insulin sensitivity. Insulin diagnostic evaluation is essential for understanding the pathophysiology of diabetes mellitus and metabolic syndrome (60). Type 1 diabetes (insulin deficiency) results from the autoimmune eradication of pancreatic beta cells, while insulin resistance (Metabolic Syndrome, Type 2 Diabetes) results from a decrease in target tissue sensitivity to insulin. Oral Glucose Tolerance Test (OGTT) assesses insulin and glucose levels before and after glucose consumption, while Hyperinsulinemic-Euglycemic Clamp is the gold standard for assessing insulin sensitivity in research settings (61). Reactive hypoglycemia or an insulinoma often induces excessive insulin secretion, with diagnostic examinations including fasting insulin and glucose concentrations, 72-hour fast, and imaging (62). Obstacles in insulin diagnostics include rapid fluctuation of insulin levels,

assay variability, diet and stress, and interpretation of insulin levels.

The anterior pituitary gland secretes ACTH, a hormone that plays a crucial role in the body's metabolism, stress response, immune function, and metabolism. ACTH is secreted in a pulsatile manner, with surges occurring every 1-2 hours (63,64). It is regulated by the circadian rhythm, with the highest concentrations observed in the early hours of the morning and the lowest levels occurring in the late evening and early morning. ACTH has two primary functions: stimulating the adrenal cortex to produce and release cortisol, a glucocorticoid that regulates metabolism, immune response, and stress response. It also stimulates the secretion of adrenal androgens and, to a lesser extent, aldosterone. Diagnosis of ACTH levels is crucial for diagnosing disorders such as Cushing's syndrome, Addison's disease, or secondary adrenal insufficiency.

Cortisol is a hormone that plays a crucial role in the body's metabolism, stress response, immune function, and metabolism. It is secreted by the anterior pituitary gland, which stimulates the adrenal cortex to release cortisol in a pulsatile manner. The secretion of cortisol is regulated by the circadian rhythm, with the highest concentrations observed in the early hours of the morning and the lowest levels occurring in the late evening and early morning. Cortisol is released in ultradian pulses, which vary in amplitude and frequency, in addition to the circadian rhythm. Cortisol diagnostic evaluation involves measuring cortisol levels to identify conditions such as Cushing's syndrome, Addison's disease, or secondary adrenal insufficiency. Hypercortisolism, primary adrenal insufficiency, and non-thyroidal illness and stress can be indicated by abnormal cortisol levels (65). Diagnostic examinations include 24-hour Urinary Free Cortisol (UFC), Late-Night Salivary Cortisol, Dexamethasone Suppression Tests, ACTH Stimulation Test (Cosyntropin Test), Adrenal Antibodies, and Secondary Adrenal Insufficiency.

Prolactin is a hormone secreted by the lactotroph cells of the anterior pituitary gland in a pulsatile manner, with surges occurring throughout the day. It is regulated by the circadian rhythm, with peak levels observed during sleep and awake hours (66). Factors for hyperprolactinemia include physiological factors such as stress, exercise, pregnancy, lactation, or nipple stimulation. Pathological causes include pituitary adenoma (prolactinoma), hypothyroidism, and chest wall trauma or surgery. Diagnosis of hyperprolactinemia involves serum prolactin concentrations, macroprolactin testing, thyroid function tests, pituitary magnetic resonance imaging (MRI), and pregnancy tests. Hypoprolactinemia is uncommon and typically asymptomatic, except for postpartum women experiencing lactation failure. Diagnostic examinations include blood sampling, macroprolactin measurement, MRI of the pituitary gland in association with suspected prolactinoma, and thyroid function exams.

CONCLUSION

To ensure a thorough examination, it is crucial to correlate pulsatile hormone levels with the patient's medical history and other test findings, in addition to establishing laboratory-wide standards for testing and reference ranges that improve diagnostic accuracy. The reliability of results and the precision of diagnoses can be enhanced by taking precautions before analysis begins to try to lessen the impact of problems encountered during this stage (such as keeping the cold chain intact and using the correct collecting tubes). New assays and a deeper understanding of hormonal dynamics will allow for the development of more accurate diagnostic tools, such as the IGF1 and IGFBP3 growth hormone tests.

Declaration by Authors

Ethical Approval: NA

Acknowledgement: The authors are thankful to the Management of Apollo Diagnostics, Global Reference Lab, Hyderabad 500037, Telangana, India for their support during the study.

Source of Funding: NA

Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: E. Maruthi Prasad, Battina Rajesh, M. Sujana Reddy, Rajesh V. Bendre. Diagnostic challenges associated with rhythmic, pulsatile hormones in humans. *Int J Health Sci Res.* 2025; 15(9):472-486. DOI: *https://doi.org/10.52403/ijhsr.20250954*

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