

## **Salivary Hormone Testing: Science, Benefits, Limitations & Clinical Applications**

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Salivary hormone assessment is emerging as a regular diagnostic component in many clinical practices, particularly those in which hormone manipulation and replacement are regularly conducted. Salivary hormone testing appeals to patients because it is less costly than blood-based testing, and is performed at-home in a non-invasive fashion.

Biotechnological advances have evolved such that the diagnostic accuracy of salivary hormone test results are measured in picomole ( $10^{-12}$ ) concentrations utilizing EIA or RIA procedures<sup>1,2</sup>.

This discussion will orient you on four key points about salivary hormone testing:

- Scientific validity
- Benefits
- Limitations
- Clinical applications

### **Scientific Literature Validates Salivary Hormone Testing**

Scientific literature has published salivary reference ranges for cortisol, DHEA-S, estradiol, progesterone, testosterone, melatonin and other hormones<sup>3, 4, 5, 6, 7</sup>. Reference ranges frequently include temporal ranges such as circadian ranges for cortisol, DHEA-S, melatonin and male testosterone, as well as monthly ranges for female estradiol, progesterone and testosterone. Salivary estradiol has gestational week ranges.

Literature reveals temporal fluctuations of hormones, such that multiple sample assessments are more valuable clinically. Researchers collect multiple samples in a 24-hour period to assess circadian patterns, or collect multiple samples over many days to assess monthly patterns. The 24-hour pattern of steroid hormones in the saliva is well defined, and studies often address diurnal pattern analysis and chronobiology<sup>8, 9, 10, 11</sup>.

When we consider the traditional recognition of circadian patterns in serum cortisol and serum melatonin tests, the need for multiple samples in saliva should not be a surprise. Diurnal and monthly patterns of hormones have always been a concern in diagnostic medicine. In the 1970s, the erratic nature of plasma progesterone was noted as a limitation of a single assay when trying to determine function of the corpus luteum<sup>12</sup>. Unfortunately, ideal multiple-sample assessment yielded to economical single measurement of progesterone. Salivary hormone assessment now provides an opportunity to properly assess steroid hormone temporal patterns.

Multiple-sample assessment is critical when assessing menopause and postmenopause hormone levels. Unstable hormonal fluctuations have been observed years after menopause<sup>13</sup>. These hormonal fluctuations may play a role in many of the symptoms of menopause. Hormonal fluctuations are associated with increased occurrences of migraine headaches, obesity, mood changes, and bleeding irregularities in perimenopause and postmenopause women<sup>14, 15, 16, 17</sup>.

### **Saliva Tests Reveal Functional Integrity of Endocrine Tissues**

The most salient feature of salivary hormone assessment is that it reflects the non-protein-bound 'free' fraction of hormones at a given point in time<sup>18</sup>. Since steroid hormones are predominantly bound to carrier proteins in the blood, the unbound (and loosely bound) fraction is considered more readily available to the cells of the body. By reflecting this bioavailable fraction of hormones<sup>19</sup>, saliva assays exhibit hormone concentrations at the tissue level at a given point in time<sup>20</sup>.

This "point in time" assessment allows temporal pattern analysis of endocrine tissues that can not practically be done with serum. Endocrine tissues have a temporal pattern. Temporal pattern analysis of

adrenal, pineal and gonadal tissues represent functional integrity of these tissues. Disruptions of the temporal pattern and lack of diurnal variation in postmenopausal women<sup>21</sup> is a cardinal sign of endocrine senescence. Once-a-day dosing of steroid hormones can restore diurnal patterns and ameliorate symptoms associated with circadian dysfunction, such as sleep and fatigue disorders. Multiple sample assessment allows proper assessment in women of menopause age by allowing the degree of fluctuations to be observed.

Additional benefits of saliva hormone assessment support temporal pattern analysis capabilities of these tests. The non-invasive nature of saliva collection does not elicit stress responses that venipuncture does, thereby reducing hormone shifts and allowing greater ease in multiple sample collection for temporal pattern analysis.

### **Saliva Tests Require Proper Collection**

Since significant changes in SHBG may affect levels of salivary hormones, therapeutic interventions that shift SHBG must be recognized for proper assessment. The carrier protein sex-hormone-binding-globulin (SHBG) is also called *Testosterone-estrogen-Binding-Globulin* (TeBG). The small “e” represents a lower affinity for binding estrogen than for testosterone. Paradoxically, the production of this protein is induced by estrogen<sup>22</sup>.

Increasing SHBG production necessitates a waiting period between the beginning of hormone replacement therapy and the collection of saliva for hormone assessment. SHBG and other carrier proteins maintain equilibrium by maintaining a *steady state* between bound and unbound levels of hormones. Premature testing after therapeutic intervention will always result in elevated salivary levels. Clinical observations suggest that steady state is achieved after one month of therapy.

Even after SHBG and other carrier proteins have reached steady state, the laws of pharmacokinetics still apply. Exogenous steroid hormones peak 4 to 6 hours after administration and will sustain therapeutic trough levels 24 hours after administration<sup>23, 24, 25</sup>. Collecting saliva during the peak or down slope will always result in elevated saliva levels. It is preferable to take saliva 24 hours after the last dose when therapeutic levels are present due to the steady state effect of carrier proteins.

Based on these observations, two rules of salivary hormone assessment are “*wait one month after initiation or change of therapy*” and “*wait 24-hours after the last dose.*” Since precursors may raise hormone levels further along the steroidogenic pathway, the ability of pregnenolone, progesterone, DHEA and androstenedione to increase estradiol (and in turn SHBG) is another consideration. So, a third rule is “*these waiting periods apply to precursors as well*”<sup>26</sup>.

### **Saliva Hormone Tests Provide Unique Clinical Insights**

Saliva tests provide clinicians with a more complete portrait of endocrine functional capacity by allowing non-invasive multiple-sample assessment of steroid hormones. Clinicians that continue to use serum assays are using these salivary portraits to understand why hormone levels are low. Temporal dysfunctions can only be revealed through temporal pattern analysis.

Multiple-sample salivary hormone tests allow for assessment of hormonal fluctuations in perimenopausal and postmenopausal women, a diagnostic tool not readily available until recently. As noted, these fluctuations play a larger role in quality of life for the menopause-aged woman. The non-invasive, economical nature of multiple sample saliva tests provides ample objective data for both baseline and therapeutic efficacy assessments.

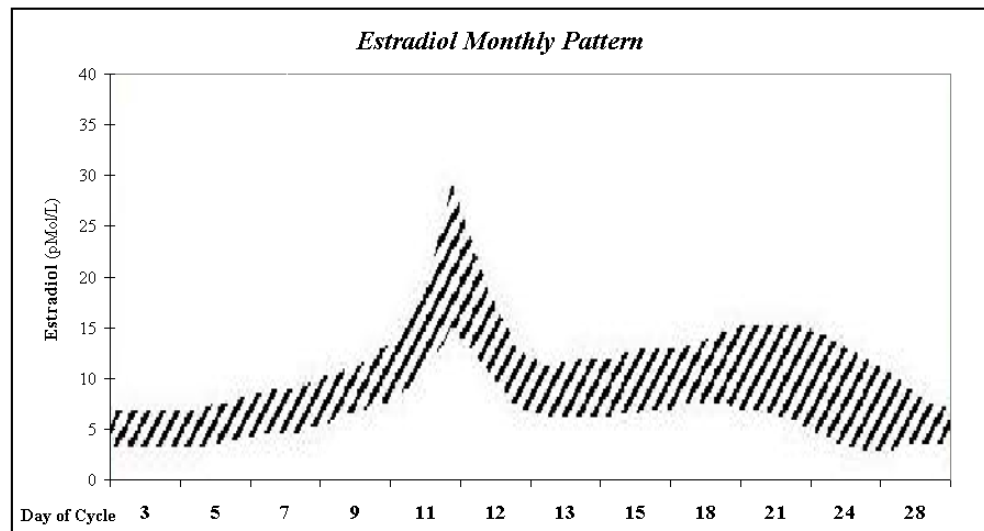
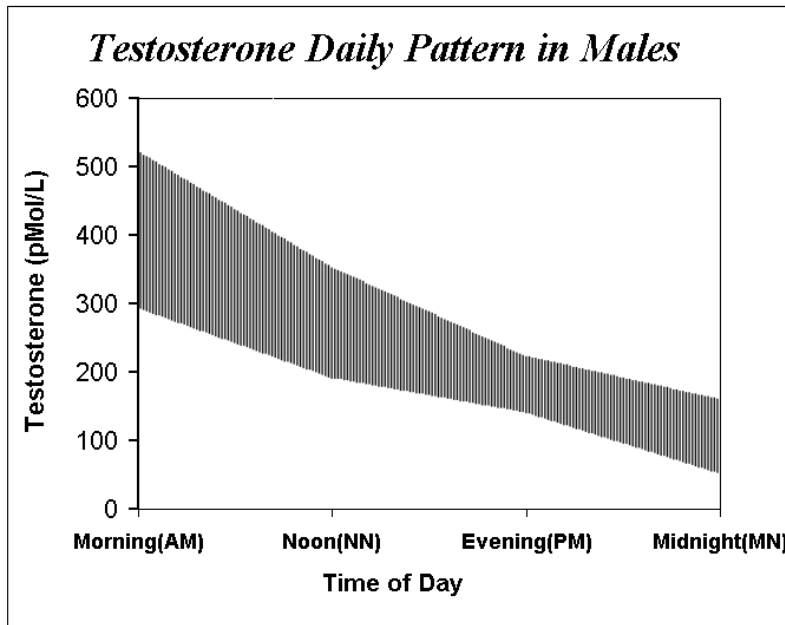
The interrelationship between hormones is clinically important. As such, these multiple-sample tests frequently include multiple markers. The functional capacity of the adrenal gland is assessed through assaying both cortisol and DHEA-S. A male hormone test includes both testosterone and estradiol, while the female cycle test includes estradiol, progesterone and testosterone. Menopause tests now include three assays each of estradiol, progesterone and testosterone.

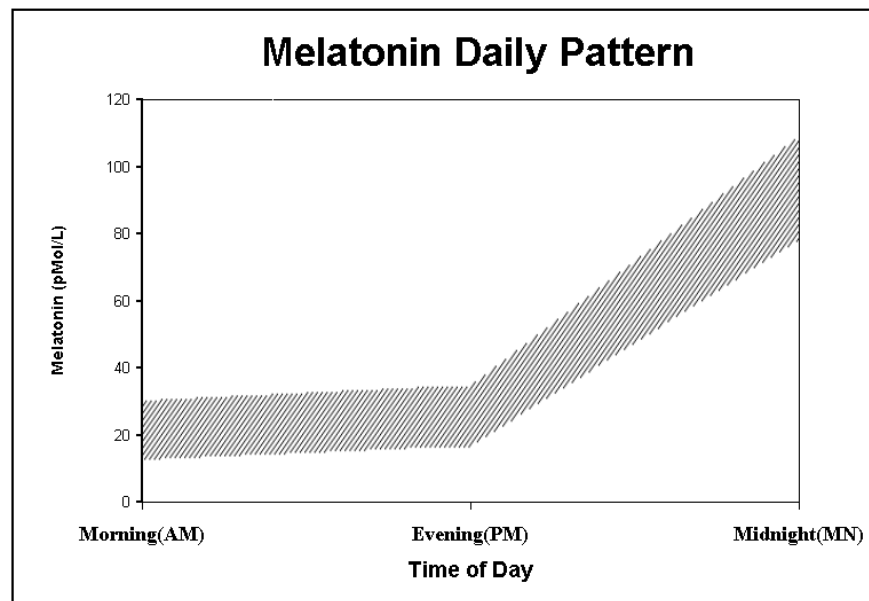
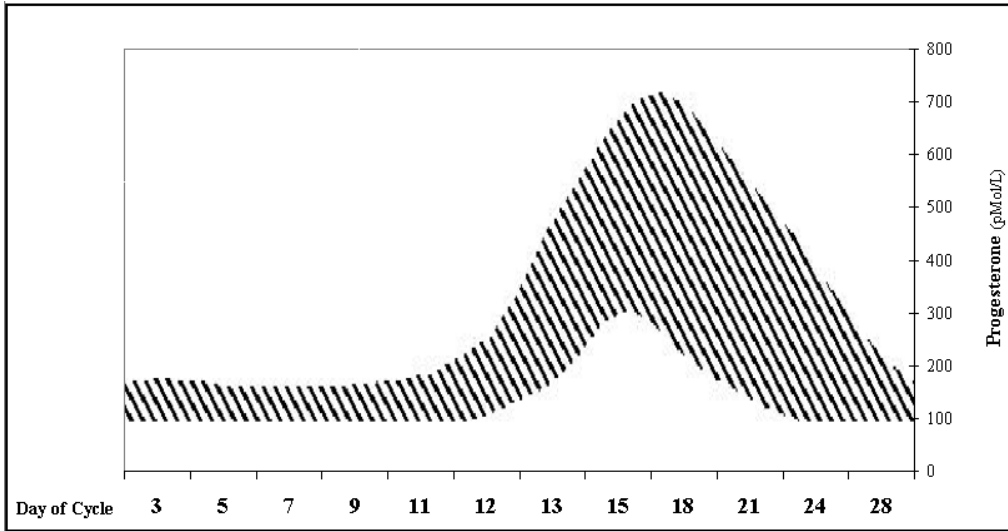
Ratio analyses of these interrelated markers provide even greater detail to the portrait of endocrine functional capacity. The DHEA-S/cortisol ratio analysis reflects shifts in the steroidogenic pathway associated with stress or panic disorders<sup>27, 28</sup>. The progesterone/estradiol ratio may provide information related to libido, mood, fertility, irregular bleeding, endometrial hyperplasia, polyps, fibromas, mammary dystrophies, and disturbance of mood, appetite and thermoregulation<sup>29, 30, 31, 32, 33</sup>.

These endocrine portraits also present the clinician with variant patterns of senescence<sup>34</sup>, and give clear direction for customized therapeutic interventions. Through variant pattern analysis twelve menopause types have been identified<sup>35</sup>. The identification of menopause type allows therapeutic differentiation based on deficiency or adequacy of estradiol, progesterone or testosterone, or on excessive testosterone.

The ability of salivary hormone tests to assess the functional capacity of endocrine tissues, demonstrate hormonal interrelations, provide ratio analysis, and reveal variant patterns of senescence provides a unique contribution to anti-aging medicine. Salivary hormone tests are a component of the LEXCORE study. The LEXCORE study is an unprecedented longitudinal study of aging and anti-aging therapeutics<sup>36</sup>.

### Temporal Pattern Analysis Resultant from Salivary Hormone Testing:





**Conclusion:**

Adrenal gland function, male hormone interrelationships, female cycle patterns and menopause types are revealed through multiple samples and multiple marker analysis of salivary steroids. Salivary assessments of hormones provide an accurate detailed portrait of endocrine functional capacity that can not be derived from serum tests alone. All indications are, that as the science, benefits, limitations & clinical applications of salivary hormone testing become more evident, this biotechnological advancement will continue to redefine how medicine is practiced.

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