



# When Saliva? *When Serum?*

Saliva testing is proving to be the most reliable medium for measuring hormone levels. Hormone levels in saliva accurately represent the amount of hormone delivered to receptors in the body, unlike serum which represents hormone levels that may or may not be delivered to receptors of the body. Clinically, it is far more relevant to test the amount of hormones delivered to the tissue receptors as this is a reflection of the active hormone levels of the body.

The majority of hormones in the blood exist in one of two forms: free (5%) or protein bound (95%). While 95% of the hormones in the body are protein bound, it is only the 5% free hormones that are biologically active. Saliva measures the free, bioavailable hormone levels in the body, while serum measures only the protein bound, non-bioavailable hormone levels. Therefore, serum is a much less accurate measurement than that of saliva when assessing functional hormone levels.

## Saliva measures the “unbound” biologically active or free hormone levels in the body:

When blood is filtered through the salivary glands, the bound hormone components are too large to pass through the cell membranes of the salivary glands. Only the unbound hormones pass through and into the saliva. What is measured in the saliva is considered the “free”, or bioavailable hormone, that which will be delivered to the receptors in the tissues of the body.

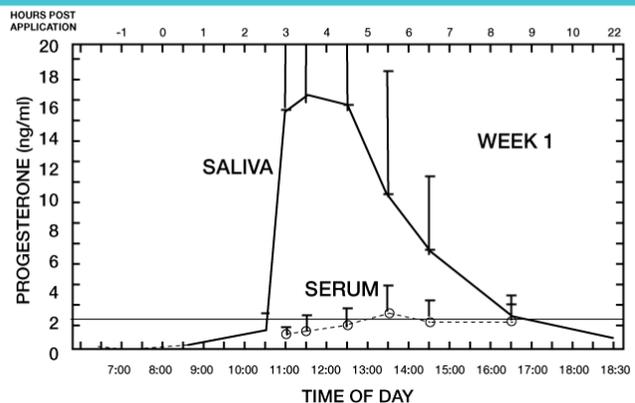
## Serum measures the “protein bound” biologically inactive hormone levels in the body:

In order for steroid hormones to be detected in serum, they must be bound to circulating proteins. In this bound state, they are unable to fit into receptors in the body, and therefore will not be delivered to tissues. They are considered inactive, or non-bioavailable.

## Only saliva testing measures topically dosed hormones:

The discrepancy between free and protein bound hormones becomes especially important when monitoring topical, or transdermal, hormone therapy. Studies show that this method of delivery results in increased tissue hormone levels (thus measurable in saliva), but no parallel increase in serum levels. Therefore, serum testing cannot be used to monitor topical hormone therapy.

### Saliva and Serum Progesterone Following Percutaneous Application of 20mg Progesterone



*Topical Supplementation can be monitored best in saliva*

# Salivary Hormone Testing

## *Bibliographies and References*

The following references are articles found by searching the peer reviewed literature that address the benefits of saliva over serum. In my searching I have not discovered one article that refutes the benefits of saliva over serum testing.

*Percutaneous Administration of Progesterone: Blood Levels and Endometrial Protection.* Stanczyk FZ, et al. *Menopause* (2005), 12(2): 232-237. A very good review of the issues related to the effectiveness of topical administration of progesterone on the endometrium and the disparity between saliva and serum levels. The red blood cell (RBC) carrier theory is validated.

*Salivary, But Not Serum or Urinary Levels of Progesterone are Elevated After Topical Application of Progesterone One Cream to Pre- and Postmenopausal Women.* O'Leary P, et al. *Clin Endo* (2005) 53: 615-620. Researchers applied 64mg of progesterone topically to 6 each pre and postmenopausal women. The continuous 3hr serum and 24hr urine (including pregnanediol-3-glucuronide metabolite) samples showed no significant level changes; whereas, remarkable elevations were noted in the saliva. Authors question clinical organ response without a measurable serum level, though organ delivery was obvious. They also suggest that the lymphatic system delivers the hormones rather than red blood cells (RBCs).

*A Study to Evaluate Serum and Urinary Hormone Levels Following Short and Long Term Administration of Two Regimens of Progesterone Cream in Postmenopausal Women.* Carey BJ, et al. *British J Obstetrics and Gynecology* (2000) 107:722-726. Authors evaluated serum and urine levels in 24 pre and postmenopausal women following the topical application of 40mg of progesterone either bid (divided dosage) or qd. Conclusion: "Transdermal progesterone (40mg) per day for 42 days causes a small increase in serum progesterone concentration, although there is wide variation. Whether such levels are of clinical benefit remain to be seen." There was no change in the metabolite.

*Topical Progesterone Cream Has an Antiproliferative Effect on Estrogen-Stimulated Endometrium.* Leonetti HB, et al. *Fertility and Sterility* (2003) 79:221-2. Authors monitored the proliferative activity of endometrial cell biopsies in 32 postmenopausal women following 0.625mg conjugated equine estrogen (CEE) and given either bid daily 0, 1.5% or 4% progesterone topically. Endometrial biopsy evaluation after 2 weeks of progesterone clearly showed an antiproliferative effect of topical progesterone. The antiproliferative effect was essentially the same for the 1.5% and 4% dosages. Regarding serum testing, the authors comment: "The plasma concentrations of progesterone were low and varied greatly among individuals. However, elevated serum levels are irrelevant, provided one obtains the desired clinical outcome."

*Micronized Transdermal Progesterone and Endometrial Response.* Wren BG, et al. *Lancet* (1999) 354: 1447-8. Authors randomized 27 estradiol exposed (Climara 100 weekly) postmenopausal women into 16mg, 32mg or 64mg groups. Serum levels and endometrial biopsies were monitored. Summary: The use of transdermal progesterone for 14 days over three cycles, even at concentrations as high as 64 mg daily, did not increase circulation blood progesterone concentrations sufficiently to induce any evidence of secretory effect in the endometrium.

*Hormones in Saliva.* Vining RF and McGinley RA. *Critical Reviews in Clinical Laboratory Sciences.* (1986) 23(2):95-146. An excellent review article about the constituents of saliva. Conclusion: "Saliva flow rate does affect saliva pH and the concentration of many salivary ions. This has led many clinicians to assume that it would also affect all salivary steroid levels. This is not the case—a number of clinically important steroids, such as cortisol, testosterone, estradiol and progesterone, have salivary concentrations which are not appreciably affected by saliva flow rate. However, the conjugated steroids (e.g., DHEAS) and some unconjugated (e.g., cortisone) steroids may exhibit marked flow rate dependence."

*Salivary Cortisol: A Better Measure of Adrenal Cortical Function than Serum.* Vining RF, et al. *Ann Clin Biochem*

(1983) 20:329-35. Prospective study: three groups (ages 24-32) consisting of 7 healthy men and women and 10 third trimester pregnant women. Advantages of saliva: reflects bio-available cortisol and is unaffected by corticosteroid binding globulin (CBG) level, which rises with birth control pill (BCP) use and during pregnancy, stress free and easy to collect, lends itself to multiple samples. Intravenous (IV) cortisol injection shows salivary rise within 5 mins. Routine serum samples at 0900 and 1700 do not accurately reflect adrenal dysfunction.

*Influences of Percutaneous Administration of Estradiol and Progesterone on Human Breast Epithelial Cell Cycle In Vivo.* Chang KJ, et al. *Fertil Steril* (1995) 63(4):785-91. Randomized placebo controlled study of 40 premenopausal women scheduled for excisional biopsy of benign lesions. Study groups were given either progesterone (Pg) 25mg or estradiol (E2) 1.5mg or both topically qd to the surgical breast 10-13 days before surgery. Findings: Both E2 and progesterone readily penetrated the skin, increasing the progesterone level x100. Progesterone induced a major reduction in the acinar cell proliferation rate whether used alone or in combination with E2. The serum levels did not reflect the topical hormone supplementation.

*Salivary Cortisol Determined by Enzyme Immunoassay is Preferable to Serum Total Cortisol for Assessment of Dynamic Hypothalamic-Pituitary-Adrenal Axis Activity.* Gozansky WS, et al. *Clin Endocrin* (2005) 63:336-341. Author compared salivary and serum cortisol levels between 12 individuals under various conditions: exercise, stress, dexamethasone suppression or corticotrophin-releasing hormone (CRH) stimulation. Enzyme immunoassay (EIA) was the salivary test method compared to serum radioimmunoassay (RIA). Conclusion: "Therefore, assessment of salivary cortisol should be considered over serum total cortisol because more physiologically relevant data are obtained, particularly when the cortisol response to a hypothalamic-pituitary axis (HPA) stimulus exceeds the saturation of corticosteroid-binding globulin (CBG)."

*Direct Assay for Progesterone in Saliva: Comparison With a Direct Serum Assay.* Webley GE, Edwards R. *Ann Clin Biochem* (1985) 22:579-585. Study compares direct serum and saliva assays for sensitivity, precision and recovery. Twenty women in various stages of their menstrual cycle were compared using serum and saliva. Conclusion: Saliva showed a significant correlation ( $r=0.71$ ,  $P<0.001$ ) compared to serum with the added advantages of convenience and reduced stress (no needles).

*Human Erythrocyte Membrane Uptake of Progesterone and Chemical Alterations.* Devenuto F, et al. *Biochem Biophys. Acta* (1969) 193:36-47. Studied red blood cell (RBC) membrane uptake of progesterone, corticosterone and cortisol in fresh and 42 day stored (blood bank) blood. Findings: progesterone showed a much greater affinity for red blood cell (RBC) constituents (6 to 8 times greater) than the glucocorticoid hormones. Furthermore, there is a likely direct relationship with the amount of bound progesterone and the viability of RBCs in storage, e.g., female blood may be more stable in storage. Also, indirectly this data supports the RBC as a carrier medium for topical applied progesterone.

*Saliva as a Medium for Investigating Intra- and Interindividual Differences in Sex Hormone Levels in Premenopausal Women.* Peter H. Gann, Susan Giovanazzi, Linda Van Horn, Amy Branning, and Robert T. Chatterton, Jr. *Cancer Epidemiology, Biomarkers and Prevention*. Vol. 10, 59-64, January 2001. Authors describe saliva's advantages: "Saliva has several advantages over blood as a sampling medium: it can be easily collected by subjects themselves at repeated intervals; it requires no special storage or collection equipment; and the steroid concentrations measured exclude the fraction tightly bound to serum proteins and thus unavailable for biological action. Most importantly, consecutive samples can be grouped for analysis after the length of the menstrual cycle is known."

*Patterns of Salivary Estradiol and Progesterone Across the Menstrual Cycle.* Gandara, B et al., *Annals of the New York Academy of Sciences*. 1098: 446-450 (2007).

*Validation of salivary testosterone as a screening test for male hypogonadism.* Morley, J et al. *The Aging Male*. September 2006; 9(3): 165-169.

*Salivary cortisol and DHEA levels in the Korean population: age-related differences, diurnal rhythm, and correlations with serum levels.* Ahn RS, Lee YJ, Choi JY, Kwon HB, Chun SI. *Yonsei Med J*. 2007 Jun 30;48(3):379-88.

*Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender.* Netherton C, Goodyer I, Tamplin A, Herbert J. *Psychoneuroendocrinology*. 2004 Feb;29(2):125-40.

*Diurnal and seasonal cortisol, testosterone, and DHEA rhythms in boys and girls during puberty.* Matchock RL, Dorn LD, Susman EJ. *Chronobiol Int.* 2007;24(5):969-90.

*Salivary Sex Hormone Measurement in a National, Population-based Study of Older Adults.* Gavrilova N, Lindau ST. *J Gerontol B Psychol Sci Soc Sci.* 2009 Nov; 64 Suppl 1:i94-105. A large study that measured salivary levels of estradiol, progesterone, DHEA and testosterone in both men and women and found them to be a reliable, minimally invasive way of adding a physiological component to in-home, population-based studies.

*Saliva Estriol Levels in Women with and without Prenatal Antidepressant Treatment.* Suri RS, Helleman G, Cohen L, et al. *Biol Psychiatry.* 2008 Sep 15;64(6):533-7. 71 women with a diagnosis of major depressive disorder (some of whom were on antidepressant therapy) and 19 women who had no psychiatric history were followed during their pregnancies and salivary estriol levels were analyzed at three different occasions during the pregnancies. The study found an association between antidepressant use and increased estriol levels in saliva during the second half of the pregnancy. Women using antidepressants gave birth at a shorter gestational age and had a higher rate of prematurity, however it is unclear whether the observed shortening of the duration of pregnancy is a result of the elevated estriol levels or caused by some other mechanism.