Progesterone

Salivary Progesterone Measurement in Wave I of the Social Life Health & Aging Project

Authors:

Bhairavi Nallanathan, University of Chicago, Department of Ob/Gyn

Karl Mendoza, BS, University of Chicago, Department of Ob/Gyn

Mary J. Curran, Salimetrics, LLC

Stacy Tessler Lindau, MD, MAPP, University of Chicago, Departments of Ob/Gyn and Medicine – Geriatrics*

* Corresponding author. Fax: +1 773 834 5664.
E-mail address: slindau@uchicago.edu (S.T. Lindau).

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Rationale

Derived from cholesterol, progesterone is a steroid hormone, found in both females and males (Andersen and Tufik 2006). In females, progesterone is produced in the adrenal cortex, corpus luteum and, during pregnancy, the placenta. In reproductive age females, the primary function of progesterone involves preparation and maintenance of the endometrium for implantation of fertilized eggs (Speroff, Glass et al. 1999). Progesterone levels are used to assess ovarian

function and defects, as well as monitoring of infertility treatments (Groschl, Rauh et al. 2001; Ishikawa, Sengoku et al. 2002).

In postmenopausal women, there is a significant decrease in progesterone levels. Because progesterone may be linked to sexuality, this decline may be related to diminished sexual function and desire (Dennerstein, Alexander et al. 2003). Increased levels of progesterone have also been found in states of stress and anxiety; this may relate to its sedative or stress-counteracting effects (Wirth and Schultheiss 2006; Wirth, Meier et al. 2007). Pharmaceutical doses of progesterone have been used to decrease sexual desire in men, however the natural, physiological role of progesterone in men has not been extensively researched (Andersen and Tufik 2006).

Measurement

Sex hormone assays, particularly in the clinical setting, are typically performed on a serum specimen (Kaufman and Lamster 2002). Salivary measures have been developed and offer a relatively convenient and minimally-invasive approach for obtaining sex hormone data (Worthman, Stallings et al. 1990; Kaufman and Lamster 2002; Granger, Shirtcliff et al. 2004). These measures are representative of active, unbound steroid concentrations in the blood (Worthman, Stallings et al. 1990; Lu, Bentley et al. 1999).

Salivary and serum progesterone concentrations are highly correlated (Bolaji 1994; Ellison and Lipson 1999; Lu, Bentley et al. 1999), In a study by Lu et al. (1997) among women aged 20-40 ($n = 48$), serum-saliva progesterone correlation, in the luteal phase of menstrual cycle, was reported to be highly correlated ($r = 0.75$ ($P < 0.001$)) (Lu, Chatterton et al. 1997). Reported salivary concentrations, in proportion to free serum concentrations, have varied from 0.82% to 2.1%. In earlier work summarized by Lu et al., saliva-serum correlations were also high: ($r = 0.75$ and 0.93) (Lu, Bentley et al. 1999). Among pre-menopausal women, a substantial rise in salivary progesterone has been reported on the day after ovulation (Lu, Bentley et al. 1999).

Materials used in the construction of collection tubes and vials (Lu, Chatterton et al. 1997) and blood contamination (Kivlighan, Granger et al. 2005) may affect salivary progesterone measurements.

Population Norms

To our knowledge, no population-based data on serum progesterone levels are available.

Table 1. Salivary Progesterone Levels in Women During Different Phases of Menstrual Cycle

Group	N	Mean (pg/mL)	SD (pg/mL)
Follicular phase*	127	80.35	34.8
Luteal phase*	202	131.00	54.5
Pre-menopausal, day 20*	23	136.30	82.3
Post menopausal, day 20**	11	58.90	29.7

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*Note: These values were taken from young adults, aged 18-30.

** These values were taken from subjects aged 50-70.

Young Adults

Table 2. Mean Salivary Progesterone Levels Measured Among Women During Different Menstrual Phases and Among Men (in pg/mL) (SEM)						
	N	Mean Age	Premenstrual Phase	Midcycle Phase	Menstrual Phase	Source
Normal-cycling women	11	22.5	88.0 (13.1)	26.4 (2.1)	27.9 (2.7)	(Schultheiss, Dargel et al. 2003)
Normal-cycling women	38	19.78	-	38 (6)	-	(Schultheiss, Wirth et al. 2004)
Women taking oral contraceptives	12	21	21.1 (1.9)	25.1 (1.8)	21.5 (1.8)	(Schultheiss, Dargel et al. 2003)
Men	12	23	22.8 (1.9)	22.6 (1.3)	24.6 (2.2)	(Schultheiss, Dargel et al. 2003)
Men	20	19.78	-	25 (1)	-	(Schultheiss, Wirth et al. 2004)

Premenopausal Women

Table 3. Salivary Progesterone Levels Measured During Luteal Phase of Premenopausal Women (Mean age 42, N = 38) (Chatterton, Mateo et al. 2005)	
	pmol/L (SEM)
Cycle 1	329 (39)
Cycle 2	517 (43)
Cycle 3	462 (81)
Mean	436 (34)

Specimen Collection

All respondents were asked to provide a salivary specimen; 90.8% (N=2,721) agreed. 2,640 respondents were able to provide a salivary specimen. This involved production of approximately 2 milliliters of saliva (unstimulated passive drool) into a small, code-labeled polypropylene collection vial via a 5-centimeter section of a household plastic straw, following procedures recommended by Salimetrics, LLC. The procedure required approximately 5 minutes. The time of last food or water consumption prior to saliva collection was recorded.

Shipping and Storage

The salivary specimens were transported from the interview to a freezer using cold packs. Salivary specimens were stored in a freezer until they were shipped. The salivary samples were shipped to the lab on dry ice according to instructions. Upon receipt at Salimetrics, specimens were stored at -80°C in lab grade freezers.

Shipping Address	Salimetrics, LLC Attn: Receiving Dept. 101 Innovation Blvd., Suite #302 State College, PA 16803 800-790-2258

Assay

(see Salimetrics Salivary Progesterone Enzyme Immunoassay Kit package insert for details http://salimetrics.com/pdf/Progesterone%20Kit%20Insert.pdf)

On day of assay, the specimens were thawed completely, vortexed, and centrifuged at 1500 x g (@3000 rpm) for 15 minutes. Clear samples were pipetted into wells. The enzyme immunoassay was conducted at Salimetrics, LLC. The assay range was > 1 pg/ml. Assays were conducted in the following priority order: 1) estrogen, 2) progesterone, 3) DHEA, 4) testosterone, 5) cotinine and underwent 2 to 3 freeze-thaw cycles:

thaw #1: sex hormone assays
thaw #2: a subset underwent repeat sex hormone testing based on quality indicators
thaw #3: cotinine assay

Table 4. NSHAP Salivary Testing Performed at Salimetrics

Test	Units	Highest Calibrator*	Lowest Calibrator*	Lower limit of sensitivity	None detected (ND) reported if value:	Interference likely if value:
Estradiol	pg/mL	64	2	1 pg/mL	<0.5 pg/mL	>320 pg/mL
Progesterone	pg/mL	2430	10	5 pg/mL	<2 pg/mL	>5x highest calibrator
DHEA	pg/mL	1000	10.2	5 pg/mL	≤2 pg/mL	>5x highest calibrator
Testosterone	pg/mL	600	6.1	1 pg/mL	≤0.5 pg/mL	>5x highest calibrator
Cotinine	ng/mL	200	0.8	0.05 ng/mL	unable to get a number value because result is too low	dilute sample x20; report >3000 if value is still high

* Calibrator values are used to adjust instrumentation by establishing the relationship (under specified conditions) between known, standard values and the values indicated by a particular measuring instrument. See package insert for calibration curve.

Scoring

Values reported in picograms per milliliter (pg/mL). Assay range ≥ 5 pg/mL.

Performance Characteristics

A. Precision

Table 5.

The intra-assay precision was determined from the mean of 12 replicates each.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
H	12	884.61	35.1	4.0
L	12	39.23	3.3	8.4

The inter-assay precision was determined from the mean of average duplicates for 12 separate runs.

Sample	N	Mean (pg/mL)	Standard Deviation (pg/mL)	Coefficient of Variation (%)
H	12	884.15	48.7	5.5
L	12	28.04	2.7	9.6

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Note: These values were taken from young adults, aged 18-30.

B. Sensitivity

The lower limit of sensitivity was determined by interpolating the mean minus 2 SD's for 20 zero standards. The minimal concentration of progesterone that can be distinguished from 0 is 5 pg/mL.

C. Correlation with Serum

The correlation between serum and saliva progesterone was determined by assaying matched samples using the DSL serum progesterone EIA and the Salimetrics HS Salivary Progesterone EIA. The correlation between serum and saliva was highly significant with r = 0.80, (n = 35). For women (n =25), r = 0.87, and for men (n = 8), r = 0.67. The conversion equation from salivary concentration to serum concentration for this particular progesterone assay is:

Serum P (ng/mL) = 0.1387*saliva P (pg/mL) + 0.2097.

D. Specificity of Antiserum

Table 6.

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity
Prednisolone	1000	0.0021
Prednisone	1000	0.0038
Cortisone	1000	0.0106
11-Deoxycortisol	1000	0.0195
21-Deoxycortisol	1000	0.0082
17-α Hydroxy-progesterone	1000	0.0723
Dexamethasone	1000	0.0014
Triamincinolone	1000	ND
Corticosterone	500	0.1924
Testosterone	1000	ND
DHEA	1000	ND
Cortisol	1000	ND
Transferrin	1000	ND
Aldosterone	1000	ND
Estradiol	1000	ND
Estrone	1000	ND
Estriol	1000	ND

ND = None detected (<0.004)

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Note: These values were taken from young adults, aged 18-30.

Quality Control (see Table 4)

Run on each EIA test plate were six (6) standard calibrators ranging from 10 pg/mL to 2430 pg/mL and two sets of high and low controls with established ranges. A sufficient number of assay kits and controls were sequestered for the project to minimize any lot-to-lot variations over the course of the study.

Subjects' saliva samples were run in duplicate (saliva pipetted into side-by-side wells) on a single EIA plate. Assay results for each subject were acceptable when the coefficient of variation (%CV) between the duplicate results (result 1 and result 2) was <15%. In instances where the %CV between duplicates was >15%, results were accepted if the absolute value between result 1 and result 2 was <10 pg/mL. Values greater than the upper assay limit of 2430 pg/mL were run on dilution to bring the OD readings within accepted range (10 pg/mL - 2430 pg/mL). In instances when a sample returned an extremely high result (5 times the highest calibrator), dilutions were made up to 12,150 pg/mL and a flag (**) and comment "interference likely" were added to the report. Samples with results < 5 pg/mL were also repeated. Values falling between 2 pg/mL and 5 pg/mL were reported and flagged (*) with the comment "below lower limit of assay". Progesterone values less than 2 pg/mL were reported as "none detected". In rare instances, repeat results where lower than initial values and did not fall within the accepted criteria (CV% <15% or difference <10 pg/mL between values 1 & 2). These data were reported with the comment "repeat affected by freeze/thaw".

Progesterone data were compiled in Excel by the testing manager and checked for accuracy by the technical supervisor before final reports were emailed. Data was supplied with corresponding assay plate number to facilitate the calculation of intra-assay and inter-assay control values.

Availability

Product Name	Salivary Progesterone Enzyme Immunoassay Kit
Manufacturer	Salimetrics LLC
Location of Manufacturer	101 Innovation Blvd., Suite 302 State College, PA 16803 USA 800-790-2258 (USA & Canada only)
Catalog No.	1-1502/1-1512, 96-Well Kit

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