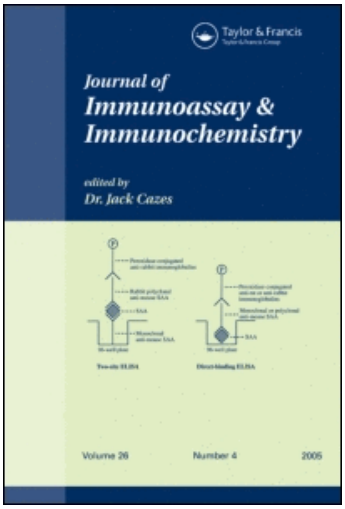

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Direct Radioimmunoassay of Progesterone in Saliva

Yu-cai Lu[a]; Robert T. Chatterton Jr.[a]; Kirsten M. Vogelsong[a]; Lynette K. May[a]
[a] Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL

To cite this Article Lu, Yu-cai , Chatterton Jr., Robert T. , Vogelsong, Kirsten M. and May, Lynette K.(1997) 'Direct Radioimmunoassay of Progesterone in Saliva', Journal of Immunoassay and Immunochemistry, 18: 2, 149 — 163
To link to this Article: DOI: 10.1080/01971529708005810
URL: http://dx.doi.org/10.1080/01971529708005810

JOURNAL OF IMMUNOASSAY, 18(2), 149-163 (1997)

DIRECT RADIOIMMUNOASSAY OF PROGESTERONE IN SALIVA

Yu-cai Lu, Robert T. Chatterton, Jr., Kirsten M. Vogelsong, and Lynette K. May
Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL 60611

ABSTRACT

We have developed a simple, direct radioimmunoassay for progesterone in saliva. The correlation coefficient (r) between the direct assay and an extraction procedure was 0.92 (n=65, P<0.001), and the correlation between concurrent serum and salivary progesterone concentrations in the luteal phases of menstrual cycles of 48 women was 0.75 (P<0.001). Whereas certain polystyrene and polyethylene vials and tubes were found to bind and remove up to 87% of the progesterone from saliva, other plastic and glass surfaces were satisfactory for the procedure. Intraassay and interassay CVs from values greater than 300 pmol/L were 12.0 and 12.4%, respectively. The assay sensitivity was 48 pmol/L. Collection of saliva is a more convenient and less invasive technique for frequent sample collection than phlebotomy, and is useful for monitoring ovulation and assessment of luteal function in women clinically.

(KEY WORDS: saliva, progesterone, human, RIA, menstrual cycle)

INTRODUCTION

Progesterone levels provide an important index of ovarian function. Clinically, measurement of serum progesterone is useful for the diagnosis and treatment of infertility patients, and for monitoring ovulation, habitual abortion and early pregnancy termination. Several investigations have shown the utility of salivary progesterone measurement for assessing luteal function and for monitoring ovulation (1-4). The noninvasive and nonstressful collection of saliva at home is an attractive alternative for patients and research subjects. The fact that samples are stable at room temperature when collected in the presence of sodium azide (4), permits daily sampling of research subjects in studies of ovarian function in clinical and epidemiological studies.

Methods have been published for the determination of progesterone in saliva, based on radioimmunoassay procedures with an extraction step (2-4), by direct assay with ^{125}I-labeled progesterone (5), or by enzyme immunoassay (6,7,8). Here we describe a sensitive, simple, direct radioimmunoassay, with ^{3}H-progesterone as the tracer, which has adequate sensitivity for salivary assays of progesterone in normal cycling women. The use of a tracer with a relatively long half-life is particularly useful when the laboratory has relatively few samples which must be assayed each month.

MATERIALS AND METHODS

Subjects:

Ten women, aged 25 to 30 years, with regular menstrual cycles (25 to 32 days), who were taking no oral contraceptives, gave daily afternoon samples of saliva through one complete cycle. Volunteers were given sugar-free gum and asked to collect 5 ml of saliva into 14 x 50 mm copolymer plastic liquid scintillation counting vials containing 0.5 mg of sodium azide. They kept the saliva samples at home at ambient temperature. After the samples were brought to the laboratory, they were stored at -20 °C until assayed. Another 48 women, aged 20 to 40 years, with regular menstrual cycles, collected saliva and blood samples at weekly intervals for four weeks. These samples were stored at -70 °C until processed for RIA. Samples were thawed at room temperature, centrifuged at 2000 x g at 4 °C for 1 hour, and the supernatant fluid was used for assay. Saliva samples lose their viscosity and were easily pipetted after they have been frozen and thawed (7).

Reagents

[1,2,6,7,16,17-^{3}H]-Progesterone (96 Ci/mmol) (^{3}H-P_4) was purchased from Amersham and stored at 4 °C. The buffers were PBS-bovine serum albumin (BSA) buffer (13.8 g NaH_2PO_4, 8.7 g NaCl, 1.05 g BSA, 0.56 g EDTA, and 0.1 g

thimerosal, per liter, pH 7.56); Gelatin buffer (4.0 g $NaH_2PO_4.H_2O$, 11.0 g Na_2HPO_4, 1.0 g sodium azide, and 1.0 g gelatin, per liter, pH 6.95); Citrate buffer (21 g citric acid monohydrate, 1 g sodium azide, 1 g gelatin, per liter, pH 3.5); and Citrate-serum buffer (1 part stripped male serum and 9 parts citrate buffer). Dextran-coated charcoal (DCC) was prepared by adding 50 mg of dextran and 500 mg of activated charcoal (Sigma Chemical Co., St. Loius, MO) to 100 ml of gelatin buffer for salivary progesterone assay and to 100 ml citrate buffer for serum progesterone assay. Steroid-free saliva or serum for preparation of standards was prepared by mixing 40 ml of male saliva or serum with 1.0 g or 5.0 g, respectively, of agarose-coated charcoal for 20 hr. (9). Progesterone was obtained from Sigma Chemical Company, St. Lous, MO. A stock solution of 1.0 mg/ml was prepared in methanol and stored at 4 °C. The range of progesterone standards was from 100 to 3200 pmol/L and a concentration of 3200 nmol/L was prepared to determine nonspecific binding (NSB) for salivary progesterone assay. Antiserum (APA-7/22/80), prepared at Northwestern Univ., was used at dilution of 1/5000. The antiserum cross reacts 12.1% with 5β-pregnanedione, 5.5% with 5α-pregnanedione, 4.2% with deoxycorticosterone, 1.6% with 20β-hydroxy-4-pregnen-3-one, 2.3% with 3α-hydroxy-5β-pregnan-20-one, 1.6% with

corticosterone, 0.6% with pregnenolone, and < 0.1% with 20α-hydroxy-4-pregnen-3-one.

Procedures

1. Extraction RIA for salivary progesterone. Fifty µl of ^{3}H-P_4 (8000 cpm in 50 µl of gelatin buffer) were added to 600 µl saliva samples in 16 x 100 mm disposable test tubes and the steroids were extracted twice with 4 ml redistilled ether. After stirring, the saliva and ether on a vortex mixer 3 times for 10 seconds each, the tubes were frozen, and the ether was decanted to another tube and evaporated. The samples were reconstituted with 600 µl PBS-BSA buffer, and 100 µl was counted to determine recovery of ^{3}H-P_4. For the assay, 200 µl of NSB and each of the standards and samples (prepared in PBS-BSA buffer) were assayed in duplicate. The same volume of PBS-BSA buffer was added to other tubes for measurement of total counts added and for total binding. Then 100 µl of the mixture of equal parts of the tracer and antibody were added to all tubes, the tubes were incubated on a reciprocal shaker at room temperature for 1.5 hours, and transferred to 4 °C for 1/2 hour. After adding 200 µl of DCC to each tube with a repeating pipettor, tubes were mixed on a rotary shaker for 10 minutes, and were centrifuged at 4 °C for 10 minutes. Aliquots of 300 µl of the supernatant were transferred to counting vials with 3.5 ml of scintillation fluid for counting.

All samples were included in one assay. Recovery of the tracer averaged 84% (range 78-93%), and the intraassay CV was 5.0%.

2. Direct RIA for salivary progesterone. Saliva samples and standards (200 µl) prepared in PBS-BSA buffer or stripped male saliva were pipetted into 12 x 75 mm culture tubes in duplicate. Then 200 µl of PBS-BSA buffer and 100 µl of the mixture of ^{3}H-progesterone and antiserum were added to each tube as described above. All tubes were incubated at room temperature for 3 hours on a reciprocal shaker and transferred to the cold room (4 °C) for 1/2 hour. Bound and unbound steroid were separated with DCC and the samples were counted as described above.

3. Direct RIA for serum progesterone. The assay was performed as described previously (10). Briefly, all samples were diluted in Citrate buffer, pH 4.0. Standards were prepared in Citrate-serum buffer. Then 100 µl of standards and serum samples were mixed with 100 µl the ^{3}H-P_4/antibody solution and were incubated at room temperature for 1.5 hours and transferred to the cold room. The rest of the procedure was the same as for salivary progesterone. The intraassay CV was 7%.

4. To determine loss of salivary progesterone due to binding to tubes and vials made of different materials, we

tested 12 x 75 mm borosilicate glass test tubes, 15 ml Corning disposable centrifuge tubes (polystyrene) and 12 x 75 mm polypropylene test tubes from Scientific Products Corp., McGaw Park, IL, as well as 20 ml glass counting vials, 12 x 75 mm polystyrene test tubes, 17 x 54 mm polystyrene tubes, 17 x 54 mm copolymer counting vials, and 20 ml polyethylene scintillation counting vials from Research Products International Corp., Mount Prospect, IL. ^{3}H-P4 was added to pooled male saliva to obtain a concentration of 9000 cpm per 0.1 ml. One ml aliquots were dispensed into each type of tube, in triplicate. Each tube contained 0.7 mg sodium azide. All samples were kept at room temperature for 24 hours, and 0.1 ml samples were removed for counting to calculate binding.

Statistical Analysis of Data

Pearson's correlation coefficients were calculated for the comparisons of direct and extraction assays and for the comparison of concomitant salivary and serum samples. Intra- and interassay coefficients (CVs) were calculated from the assay data.

RESULTS

In a comparison of standards which were prepared in stripped male saliva or in PBS-BSA buffer, consistently lower counts were obtained from those without saliva (Figure 1).

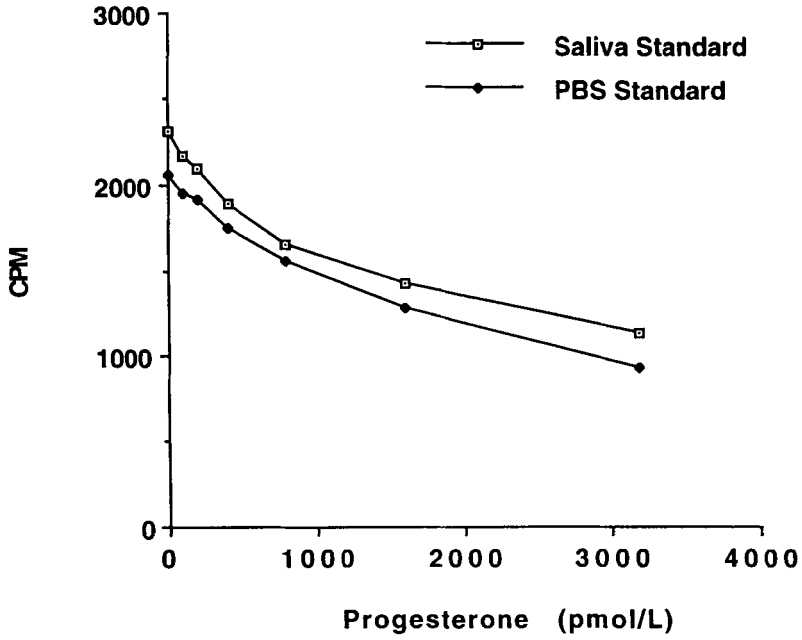

FIGURE 1. Comparison of the counts with standards made in saliva and in PBS-BSA buffer.

Therefore, all results reported were obtained from assays in which standards were prepared in saliva

The percentage of progesterone lost from binding to various tubes and vials after 24 hours is presented in Table 1. The 15 ml Corning disposable polystyrene centrifuge tubes were particularly bad with 86.7% of the tracer lost due to binding by the tube in 24 hrs.

Using the direct RIA method, mean progesterone concentrations (mean $\pm$ SE) in saliva during the menstrual

TABLE 1

Comparison of Progesterone Loss After 24 Hour Incubation of Saliva Samples in Different Containers at 23 °C (Mean ± SE percentage)

Composition of Container	Percentage Loss
Glass Tube (12 x 75 mm)	6.2 ± 1.3
Glass Counting Vial (20 ml)	7.6 ± 2.0
Polystyrene Tube (12 x 75 mm)	10.5 ± 0.8
Polystyrene Vial (17 x 54 mm)	4.9 ± 1.9
Corning Disposable Polystyrene Centrifuge Tube (15 ml)	86.7 ± 0.4
Polypropylene tube (12 x 75 mm)	9.9 ± 0.5
Copolymer Counting Vial (17 x 54 mm)	0.6 ± 0.3
Polyethylene Counting Vial (20 ml)	59.6 ± 1.8

cycles of 9 of the 10 women in the study are shown in Figure 2. One of the 10 subjects had no increase in salivary progesterone during the second half of the cycle, and was not included in this summary. Progesterone concentrations (mean ± SE) in saliva during the follicular phase of the cycle for the 9 apparently ovulatory women averaged 193 ± 24 pmol/L (range 102-330 pmol/L). The corresponding value for the luteal phase was 299 ± 70 pmol/L (range 119-702 pmol/L). The intraassay CV from values greater than 300 pmol/L was 12% and the interassay CV was 12.4%. The sensitivity (2 SD from the mean of 10 assays of stripped male saliva) was 48.2 pmol/L. A comparison of the direct and extraction assays in

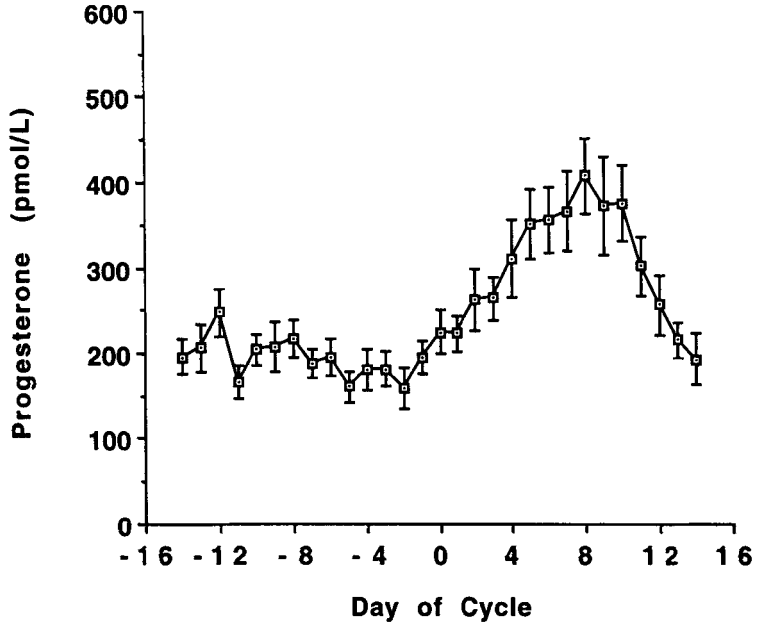

FIGURE 2. Progesterone concentrations in daily saliva samples from nine women throughout the menstrual cycles (Mean ± SE). The days of the cycle are numbered relative to the day of ovulation (day 0).

65 saliva samples gave a correlation coefficient (r) of 0.918 which is highly significant ($p<0.001$). The equation of the regression line is $Y=1.12X-55.2$, indicating that the values for the direct assay were approximately 12% higher than the values for the extraction assay.

In comparing the direct salivary and serum progesterone assays, a correlation coefficient of 0.75 ($P<0.001$) was found for single luteal phase samples from 48 women.

DISCUSSION

In preparation for this study, we investigated progesterone binding to different tubes and vials. Progesterone loss from saliva after incubation in these vessels for 24 hours was highly variable, depending on the composition of the tube or vial. Based on the data of this study, we strongly recommend testing the vials to be used, before initiating saliva collection.

As shown in this study, one of the requirements of this assay is the preparation of the standards in stripped male saliva. Since the concentrations of salivary progesterone are quite low, a sensitive assay is required. Small differences in the properties of the standards significantly influence the results of the assay. In comparing standards prepared in either saliva or PBS-BSA buffer (Figure 1), we found that the value of salivary progesterone obtained when the PBS-BSA buffer was used to prepare the standards was significantly lower than that obtained when stripped saliva was used. This would cause the progesterone values of most samples in the follicular phase, which are nearly 200 pmol/L, to be nondetectable.

One of the major advantages of measuring steroids in saliva is the ease and convenience of collecting serial samples, which provides much greater accuracy in assessing endocrine function than does a single sample (1). Daily saliva samples are easily collected by the patients themselves at home, stored

TABLE 2

Comparison of Salivary Progesterone Concentrations in Normal Menstrual Cycles (pmol/L, mean ± SD or range)

Author	Number of Subjects	Salivary Progesterone Follicular Phase	Luteal Phase
Present Study	9	193 ± 66	299 ± 112
Bourque et al. (5)	14	103 ± 57	428 ± 238
Choe et al. (2)	9	740 ± 44	1473 ± 123
Tallon et al. (7)	11	50 to 235	312 to 852
Zorn et al. (9)	32	133 ± 42	355 ± 126
Cedard et al. (3)	14	133 ± 42	339 ± 114

at room temperature and brought to the laboratory. Additionally, salivary steroid assays provide an estimate of the concentration of unbound steroids in plasma (11,12).

The direct radioimmunoassay described here is relatively simple compared to the ether extraction procedure (2-4), and the data compare well with previously published values from the several types of assays as shown in Table 2. The use of ^{3}H-P4 may have an advantage over the ^{125}I-P_4 tracer (5) for some laboratories. Since the useful half-life of ^{125}I-P_4 is only about 6 weeks, it is not convenient for a research laboratory that assays only a few samples per month. The tracer must be replaced frequently with only a small proportion being used

for the assays. Moreover [^{3}H] provides less radioactivity exposure than [^{125}I] to the staff working in the laboratory. Relative to the EIA procedures (6-8), the direct RIA requires less preparation, and, in our hands, has greater precision. It is also possible to analyze samples that have been collected with sodium azide as the preservative in the RIA but not the EIA because sodium azide is an inhibitor of the enzyme used in the published EIAs.

Reprint requests: Robert T Chatterton, Ph.D., Director, Reproductive Endocrinology Laboratory, Department of Obstetrics and Gynecology, Northwestern University Medical School, 333 E Superior St. Chicago, IL 60611

REFERENCES

1. Riad-Fahmy D., Read G.F., Walker R.F., Griffiths K. Steroids in Saliva for Assessing Endocrine Function. Endocr Rev. 1982; 3: 367-95.

2. Choe J.K., Khan-Dawood F.S., Dawood M.Y. Progesterone and Estradiol in the Saliva and Plasma During the Menstrual Cycle. Am. J. Obstet. Gynecol. 1983; 147: 557-62.

3. Cedard L., Janssens Y., Tanguy G. Zorn J.R. Radioimmunoassay of Plasma and Salivary Progesterone During the Menstrual Cycle. J. Steroid Biochem. 1984; 20: 487-90.

4. Ellison P.T. Measurements of Salivary Progesterone. Ann. N.Y. Acad. Sci. 1993; 694: 161-76.

5. Bourque J., Sulon J., Demey-Ponsart E., Sodoyez J.C., Gaspard U. A Simple Direct Radioimmunoassay for Salivary Progesterone Determination During the Menstrual Cycle. Clin. Chem. 1986; 32: 948-51.

6. Arnstadt K.I., Berg D. Sensitive Enzyme-Immunoassay for Progesterone in Human-Plasma and Saliva. J. Clin. Chem. Clin. Biochem. 1981; 19: 602-3.

7. Tallon D.F., Gosling J.P., Buckley P.M. et al. Direct Solid-Phase Enzyme Immunoassay of Progesterone in Saliva. Clin. Chem. 1984; 30: 1507-11.

8. O'Rorke A., Kane M.M., Gosling J.P., Tallon D.F., Fottrell P.F. Development and Validation of a Monoclonal Antibody Enzyme Immunoassay for Measuring Progesterone in Saliva. Clin. Chem. 1994; 40: 454-8.

9. Stockhill, C. Preparation of Thyroxine (T4) and Triiodothyronine (T3) -Free Serum Avoiding Charcoal Fines. Ann. Clin. Biochem. 1979; 16: 275.

10. Chatterton R.T., Jr., Kazer, R.R., Rebar R.W. Depletion of Luteal Phase Serum Progesterone During Constant Infusion of Cortisol Phosphate in the Cynomolgus Monkey. Fertil. Steril. 1991; 56: 547-54.

11. Vining R.F., McGinley R.A., Symons R.G. Hormones in

Saliva: Mode of Entry and Consequent Implications for Clinical Interpretation. Clin. Chem. 1983; 29: 1752-6.

12. Vining R.F., McGinley R.A., Maksvytis J.J., Ho K.Y. Salivary Cortisol: A Better Measure of Adrenal Cortical Function Than Serum Cortisol. Ann. Clin. Biochem. 1993; 20: 329-35.