

# PROGESTERONE METABOLITES

## PREMENOPAUSE

### Urinary Progesterone Metabolites in Premenopausal Women Not Supplementing with Progesterone

The urinary progestogen metabolites included in our profiles encompass the primary urinary metabolite, pregnanediol (Pgdiol), and four other more minor metabolites that belong to the pregnane (Allo-pregnanolone, Allo-pregnanediol) and pregnene ( $3\alpha$ -dihydroprogesterone,  $20\alpha$ -dihydroprogesterone) categories. In postmenopausal women the level of pregnanediol is expected to be much lower than in premenopausal women (mean values 81 and 1324  $\mu\text{g/g}$  creatinine, respectively). The mean and range levels for urinary pregnanediol established in premenopausal women during the early follicular and mid-luteal phases of the menstrual cycle are 152  $\mu\text{g/g}$  creatinine (range 92-346) and 1324  $\mu\text{g/g}$  creatinine (range 849-1932), respectively. Thus, about a 10-fold increase in Pgdiol is expected during the progression from the follicular to the luteal phase of the menstrual cycle. The urinary ranges of pregnanediol during the luteal phase are equivalent to a range of about 3-25 ng/mL progesterone in blood (capillary whole blood and venous serum or plasma) and 50-250 pg/mL in saliva. Optimal luteal ovarian production of progesterone is reflected in all three body fluids (urine, blood, salivary), which is roughly  $> 1300 \mu\text{g PgDiol/g creatinine}$  in urine,  $> 10 \text{ ng Progesterone/mL}$  in blood, and  $> 100 \text{ pg Progesterone/mL}$  in saliva.

#### OPTIMAL PREGNANEDIOL/ESTRADIOL RATIO:

Based on the optimal luteal levels of urinary Pgdiol (about 1300-2000  $\mu\text{g/g}$  creatinine) an optimal working

range for the ratio of this progesterone metabolite to estradiol was established based on the median level of urinary estradiol (1.37  $\mu\text{g/g}$  creatinine). Thus the optimal working ratio of urinary Pgdiol to estradiol in the premenopausal woman during the peak luteal phase should be in the neighborhood of about 1000 to 1500. A lower ratio, associated with higher estrogens and symptoms of estrogen dominance, is commonly seen in women approaching menopause (perimenopausal) and is often successfully treated by lowering the estrogen level with improved diet, exercise, and nutritional supplements that increase estrogen elimination, and/or by increasing progesterone with supplementation.

**IMPORTANT NOTE:** Topical progesterone raises urinary Pgdiol very little even with pharmacological dosing (50-300 mg), likely because topically delivered progesterone is excreted primarily in bile/feces. In sharp contrast, oral progesterone therapy raises urinary Pgdiol to levels much higher than seen in premenopausal women (luteal phase), without raising blood, salivary, or tissue levels of progesterone very much. For these reasons, we suggest for those individuals using oral, topical, or vaginal progesterone to evaluate the active bioavailable levels of progesterone in saliva or capillary blood (not venipuncture serum).

In addition to Pgdiol, four other progesterone metabolites are tested, which are listed above.

Research, mostly in tissue culture with breast cancer cells and a few animal models<sup>1</sup>, with progesterone and pregnene and pregnane metabolites has revealed that breast cancer cells have surface membrane receptors that are distinct from the classic intracellular progesterone receptors (A and B subunits) that bind to progesterone and activate unique gene sites associated with controlling estrogen-stimulated cell proliferation and cell differentiation. In contrast to progesterone itself, the pregnane class of progesterone metabolites, which are formed mostly from 5 $\alpha$ -reductase metabolism (the same enzyme class that converts testosterone to its more active metabolite, 5-alpha dihydrotestosterone), activate distinct surface membrane receptors and stimulate cell proliferation, increase invasive potential, and inhibit apoptosis (necessary for differentiation). In sharp contrast, the pregnene class of progesterone metabolites, represented in the progesterone metabolite profile as 20 $\alpha$ - and 3 $\alpha$ -dihydroprogesterone, have the opposite effect to inhibit cell proliferation, decrease invasion and angiogenesis, and promote cell differentiation (apoptosis). According to the theory, which is based only on in vitro studies in human breast cancer cells in culture, and some animal models of breast cancer, excessive production of the pregnane metabolites, relative to the pregnene metabolites, would favor tumor growth (assuming a breast tumor is present), whereas higher levels of the pregnene metabolites would inhibit tumor growth. One of the pregnane metabolites of progesterone is allopregnanolone, which is well studied and a known anxiolytic neurosteroid that binds to brain GABA receptors, resulting in a calming effect. In fact, higher levels of this progesterone metabolite are associated with a lower incidence of PMS in premenopausal women and better sleep patterns in both premenopausal and postmenopausal women supplementing with exogenous oral or topical progesterone. The

pregnane-mediated metabolism of progesterone via the 5 $\alpha$ -reductase is increased during pregnancy and is induced by estradiol. The 5 $\alpha$ -reductase enzyme is also much higher in breast tumors. Not unexpectedly, more of this anxiolytic progesterone metabolite is formed with oral progesterone supplementation, and less so with topical progesterone. In some individuals who convert large amounts of progesterone to allopregnanolone, the sleep-inducing effect can be overwhelming. Therefore, if progesterone causes excessive drowsiness, you are likely a high pregnane metabolizer, and it is best to use it only before bed.

Therefore, knowing the relative direction of progesterone metabolism (pregnene or pregnane formation) provides a guide to potential risk of evaluating progesterone levels in premenopausal or postmenopausal women not using progesterone, and in women using exogenous progesterone (mostly oral because topically applied progesterone is not excreted in urine). Women who are at increased risk of developing breast cancer (mid forties and older, high estrogens relative to progesterone, high stress levels and high cortisol, poor diet, and sedentary lifestyle), should evaluate how they metabolize progesterone (pregnene or pregnane pathways) before considering using the pharmacological progesterone doses (> 50 mg) often prescribed. Neither oral nor topical progesterone therapy have been shown to be associated with higher breast cancer risk; however, in women who have had breast cancer and are at risk of recurrence, progesterone supplementation should be used judiciously and pregnene and pregnane metabolism pathways examined in urine.

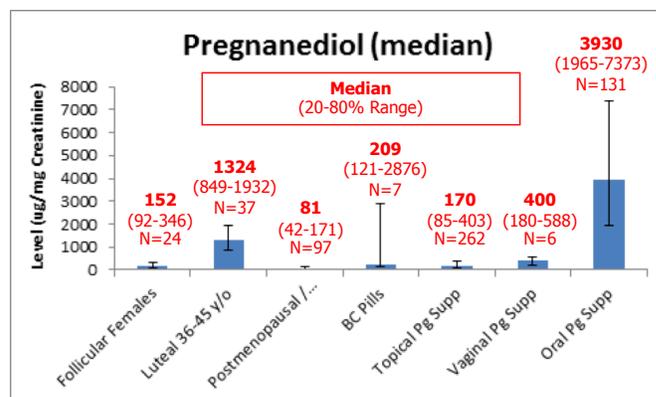
## Guide to Evaluating Luteal Function with Progesterone Metabolites in Premenopausal Women

Pregnanediol is the primary metabolite of progesterone, and women who are making adequate levels during the menstrual cycle should be making at least the median level reported (i.e., about 1300-2000  $\mu\text{g/g}$  creatinine pregnanediol) for optimal health. A higher luteal level of progesterone, which is reflected in higher Pgdiol levels, has been associated with lower breast cancer risk<sup>2</sup>.

As regards the risk of the pregnane category of progesterone metabolites potentially stimulating occult breast cancer cells, it is important to know the relative concentrations of the pregnane and pregnene metabolites as well as the relative contribution of other hormone-related breast cell growth agonists (e.g., estradiol) and antagonists (e.g., testosterone, progesterone) simultaneously present. Pregnene metabolites of progesterone compete with the more dangerous pregnane metabolites and reduce the potential growth stimulation of occult tumors. Equal concentrations of both pregnene and pregnane metabolites would have less harmful effects, but an excess of the pregnane metabolites should elicit caution against the use of progesterone in women who have had breast cancer, or who are at high risk for developing it.

**IMPORTANT NOTE:** Urinary pregnanediol results will not accurately represent the circulating levels of progesterone with the use of the following: 1) synthetic progestins in conventional hormone replacement therapy, or birth control pills; 2) topical progesterone; 3) oral progesterone; 4) vaginal progesterone. See Chart right.

Synthetic progestins are potent progestogens, bind intracellular progesterone receptors, and inhibit estrogen-activated cell proliferation in the uterine lining. However, they are not detected by immunoassays, or monitored by most methods



of mass spectrometry. While these are clinically effective, little is known about their metabolite profiles. When topical progesterone is used at physiological (10-30 mg) or pharmacological (> 50 mg) dosing, very little Pgdiol or other metabolites are found in urine or venipuncture serum (see Chart below). The small increase in urinary progesterone metabolites seen with topical progesterone use may be due to release of progesterone into the salivary glands and eventually in the gastrointestinal tract, where it would be converted to progesterone metabolites that find their way into urine, as with oral progesterone dosing. In stark contrast to urinary progesterone metabolites, physiological dosing (10-30 mg) with topical progesterone results in very high levels of salivary progesterone (range 300-3000 pg/mL, physiological range 50-300 pg/mL) but physiological levels of capillary blood progesterone (20-40 ng/mL). With topical physiological dosing progesterone is also found in physiological (luteal) levels in breast tissues in humans, and this dose of progesterone has been shown to inhibit in vivo estrogen-activated proliferation in human breast<sup>3,4</sup> and uterine epithelial cells. Thus, while urinary progesterone metabolites are a convenient way to evaluate overall endogenous ovarian production of progesterone in a premenopausal woman, this method will lead to inaccuracies in tissue uptake of exogenous progesterone supplementation, both topical (underestimation) and oral (overestimation).

## References

1. Wiebe JP. Progesterone metabolites in breast cancer. *Endocr Relat Cancer* 2006;13:717-738.
2. Micheli A, Muti P, Secreto G, et al. Endogenous sex hormones and subsequent breast cancer in premenopausal women. *Int J Cancer* 2004;112:312-318.
3. Chang KJ, Lee TT, Linares-Cruz G, Fournier S, de Lignieres B. Influences of percutaneous administration of estradiol and progesterone on human breast epithelial cell cycle in vivo. *Fertil Steril* 1995;63:785-791.
4. De Boever J, Verheugen C, Van Maele G, Vanderkerckhove D. Steroid Concentrations in Serum, Glandular Breast Tissue, and Breast Cyst Fluid of Control and Progesterone-Treated Patients. In: *Endocrinology of Cystic Breast Disease*, edited by A. Angeli, New York:Raven Press, 1983, p. 93-99.

