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WHITE PAPERS

Progesterone Therapy in Menopause

Best Practices for Selecting and Monitoring for Endometrial Health

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INTRODUCTION

EXECUTIVE SUMMARY

Hormone therapy (HT) optimization in your postmenopausal patients requires an evidence-based approach in order to maximize benefits and minimize risks associated with available therapies. This white paper provides a thorough review of clinical studies on progesterone (P4) treatment options in menopause in the context of safeguarding endometrial health. Laboratory testing options to guide therapeutic decision-making will also be discussed.

KEY FINDINGS

Dosing/Treatment

- Postmenopausal women with a uterus who are taking estrogen have to also take P4 (or another progestogen) to protect the endometrium from hyperplasia.
- Synthetic progestins can be effective at protecting the endometrium but carry risks without any additional benefits, hence their use it not recommended.
- Numerous clinical studies demonstrate that oral micronized progesterone (OMP) is safe and effective in protecting the endometrium from hyperplasia (100 mg—200 mg).
- Vaginal P4 (100 mg—200 mg) is a safe alternative to OMP for endometrial health.
- Transdermal P4 (applied to the skin) does NOT offer adequate endometrial protection against hyperplasia and should not be endorsed as a therapeutic agent to balance estrogenic effects on endometrial tissue.

Monitoring Endogenous Progesterone Levels (NOT on HT)

- Lab testing can be effective to assess endogenous P4 levels in women.
- Serum is the gold standard for measuring endogenous P4.
- Commercial saliva tests produce highly variable results and show poor correlation with results obtained from serum analysis. Saliva testing should not be considered for measurement of endogenous P4 until a commercial lab assay demonstrates serum correlation in peer-reviewed studies (none are available at this time).
- DUTCH P4 metabolites demonstrate excellent correlation with serum levels for women not on P4 HT.

Monitoring Progesterone Levels for Women on P4 HT

- Lab testing is not useful for assessing oral or vaginal P4 therapy for endometrial adequacy.
- Serum or saliva testing does not produce clinically meaningful results for monitoring OMP due to variability in pharmacokinetic distribution.
- DUTCH offers some insight into OMP's sedating and anxiolytic effects of P4 therapy, but not its impact on endometrial health.
- Lab testing does not reflect endometrial tissue P4 levels when a woman is on P4 HT and therefore should not be used for that purpose.

A healthy endometrium requires strictly controlled dynamic interplay between progesterone (P4) and estrogen. When dysregulation in hormonal signaling occurs, endometrial pathology is likely to manifest. Deepening the understanding of normal P4 and estrogen signaling juxtaposed with aberrant hormone-dependent physiological events

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ABBREVIATIONS USED

CEE	Conjugated Equine Estrogens
OMP	Oral Micronized Progesterone
ET	Estrogen Therapy
P4	Progesterone
HT	Hormone Therapy
WHI	Women's Health Initiative
WHI	Medroxyprogesterone Acetate

introduces a window of opportunity for practitioners to develop treatment plans geared to maximize health benefits of hormone therapy (HT) for their patients.

The goal of this white paper is to summarize findings from peer-reviewed, well-performed clinical studies that provide insight into targeted, efficacious, and safe HT options with P4 to achieve optimal clinical impact in menopause. Types of P4 therapies and dosing regiments will be discussed in the context of supporting endometrial health to promote longevity of clinical solutions.

A GLANCE AT ENDOMETRIAL DYNAMICS

Endometrium lines the uterus and is arguably one of the most dynamic tissues in adults, perpetually engaging in a hormonally driven cycle of transformation. Endometrial layers continuously repair, proliferate, and then shed to maintain active reproductive functions (Fig. 1).

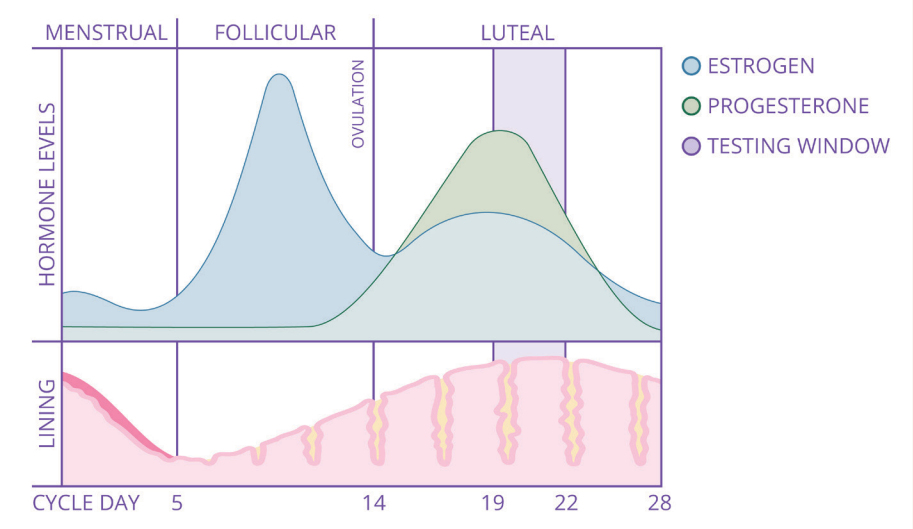

Figure 1: *Schematic representation of hormone-driven endometrial changes throughout the menstrual cycle.* **[3]**

Estrogen heralds the follicular phase of the menstrual cycle, facilitating tissue repair and endometrial proliferation **[1]**. P4 drives the luteal phase by inducing secretory differentiation, essentially thickening the endometrium to render it receptive for embryo implantation **[2]**. If ovulation takes place, P4 levels peak during the mid-luteal phase and decline in the late luteal phase in the absence of implantation, ushering in menstruation. And the cycle begins anew.

COOPERATION IS KEY!

Mutual cooperation of estrogen and P4 is paramount to achieving endometrial homeostasis – during reproductive years and in menopause. If the balance in the hormonal milieu is distorted, pathology can arise **[3]**. A prominent example of such an event is endometrial hyperplasia.

USEFUL NOMENCLATURE

Progestogen

Any bioactive molecule that can mimic endogenous P4 produced by the body in the luteal phase of the menstrual cycle. Progestogens can be synthetic (with distinctive biological and clinical profiles), or natural, such as micronized P4.

Progestins

Synthetic progestogens, subclassified into pregnanes, estranes, and gonanes, based on generation or structural properties. Depending on type, progestins can exhibit estrogenic, antiestrogenic, androgenic, antiandrogenic, antimineralocorticoid, antigonadotropic, or glucocorticoid activity.

Progesterone

Chemically and structurally identical to human P4, progesterone in HT is derived from plants and is considered 'body identical' or 'natural'. It is often called "micronized progesterone", referring to the process of micronization (making the particle smaller), which increases its absorption and bioavailability.

HORMONE IMBALANCE IN ENDOMETRIAL DISEASE

Characterized by disordered proliferation of the endometrium, endometrial hyperplasia often emerges as a consequence of hormonal imbalance–estrogen levels being too high (endogenous during reproductive years, or exogenous due to HT) relative to P4 levels being too low **[4]**. Consequently, over time, without the counterbalancing effects of P4, unopposed estrogenic activity has the propensity to give rise to endometrial malignancy. If caught and corrected early, the progression of endometrial hyperplasia to cancer can be prevented **[5]**.

INTERNATIONAL GUIDELINES

International experts agree that HT with estrogen treatment ought to be balanced by a progestogen in order to protect the endometrium and prevent endometrial disease **[6]**. What this means is, in peri- and postmenopausal women with an intact uterus, HT that includes estrogen therapy must include treatment with progesterone-like effects as well, to safeguard endometrial health.

WHICH PROGESTOGEN TREATMENT IS RIGHT FOR MY PATIENT?

Optimizing HT for your patients demands a deep awareness of the risks and benefits associated with the available progestogen therapies. Thankfully the medical community is now embracing a balanced and evidence-based approach to HT management.

This begs the question–with many synthetic progestin options available in parallel with bioidentical, i.e. natural P4, for widespread use in HT **[7]**, how do you select the right one for your patients?

A GOOD PLACE TO START

Starting with estrogen and P4 therapies that have the safest profile with respect to endometrial health, while simultaneously considering patient-specific needs, risk factors, and treatment goals is your best bet.

A deep dive into previously published peer-reviewed clinical findings offers straightforward guidance for factors to consider when selecting progestogen type and dosing **[8]**:

- **In the short term —** optimal dose should inhibit mitotic activity and therefore endometrial proliferation (≤ 2 mitoses/1,000 glandular cells at the end of a single course of treatment).
- **In the long term —** daily dose should prevent endometrial hyperplasia (≤ 2 cases of hyperplasia/100 women/year).
- **Duration of use —** therapy should maintain the desirable safety and efficacy profile throughout the entire treatment.

Lastly, although the main indication of progestogen use during HT is to prevent endometrial hyperplasia and eliminate the risk of endometrial cancer, it is important to remember that progestogenic activity expands far beyond reproductive functions in the body. What this means is that it is also critical to bear in mind breast health, the cardiovascular system, and the central nervous system, when choosing the right treatment for your patients.

ORAL MICRONIZED PROGESTERONE PROTECTS THE ENDOMETRIUM

While it is not reasonable (or possible) to assess mitotic activity in the endometrium of every patient who is ready to start HT, turning to well-performed peer-reviewed clinical studies offers evidence-based insight as to what treatment options have the best outcomes with regard to safety and efficacy profiles of HT regimens for postmenopausal women.

Lane and colleagues (1983) first demonstrated that oral micronized progesterone (OMP), combined with estrogen therapy, induced the necessary protective morphological and biochemical changes in a dose-dependent manner within the postmenopausal endometrium. The study reported that no endometrial hyperplasia was detected in postmenopausal patients who received 1.25 mg conjugated equine estrogens (CEE) daily combined with 200 or 300 mg OMP **[9]**.

After the Lane study was published, OMP has become the subject of many clinical studies, interrogating its efficacy and safety (Table 1). Its pharmacological properties have been scrutinized over the last 4 decades to show repeatedly just how effective this treatment option is for postmenopausal patients seeking relief from unwanted symptoms, while harnessing the protective nature of this treatment over the endometrial landscape. Numerous clinical studies confirmed the use of OMP in HT to achieve endometrial protection from proliferative estrogen effects as a safe and efficacious option in postmenopausal women. The overall agreement among many clinical researchers is that OMP protects the endometrium **[10] [11]**.

Further review of clinical literature provided published evidence that OMP is not only a safe and effective progestogen therapy option, but it is associated with lower cardiovascular, thromboembolic, and breast cancer risks compared with other progestogens **[12]**.

ORAL MICRONIZED PROGESTERONE IS SAFE FOR BREAST HEALTH

Not surprisingly, a question that consistently arises in the context of HT is related to the risk of breast cancer. In 2013, the Women's Health Initiative (WHI) study reported increased risk and incidence of breast cancer in women taking CEE with a synthetic progestin (medroxyprogesterone acetate, MPA), but not in the estrogen-only treatment group **[13]**. Since then, HT has received much negative publicity, stemming from misinterpretation of the WHI findings.

It is important to emphasize that women taking estrogen-only HT do not have an increased risk of breast cancer, and it is only when a progestin is added to the HT regiment that breast cancer risk ought to be evaluated. Furthermore, different types of progestogens have different types of effects on breast health and breast cancer risk. Synthetic progestins, such as MPA, may be associated with an elevated risk of developing breast cancer. In contrast to MPA, however, OMP does not increase cell proliferation in breast tissue in post-menopausal women **[14]**.

Moreover, a recent systematic review by Stute and colleagues (2018) gathered findings from a multitude of peer-reviewed studies that provide practical insight with respect to breast cancer risk–HT that combines estrogens with OMP does not increase the risk of breast cancer for up to 5 years of treatment duration **[15]**. After 5 years of use, there may be a slight increase in risk.

BREAST CANCER RISK

Synthetic progestins — associated with increased risk of developing breast cancer.

Oral micronized progesterone is safe — does not increase breast cancer risk in the first 5 years of use.

	AUTHOR	TYPE OF ESTROGEN TX	TYPE OF PROGESTERONE TX	ENDOMETRIAL PROTECTION
ORAL MICRONIZED P4	Di Carlo, et. al. 2010	50 mcg / day transdermal estradiol	100 mg–200 mg OMP for 12 days each month	Successful
	Gillet, et. al. 1994	1.5 mg / day percutaneous estradiol **OR** 3 mg / day percutaneous estradiol	100 mg OMP for 21 or 25 days **OR** 300 mg OMP for 10 days	Successful
	Judd, et. al. 1996 (PEPI study)	0.625 mg / day CEE	200 mg OMP for 12 days	Successful
	Lane, et. al. 1983	1.25 mg / day CEE	200 mg–300 mg OMP for 10 days	Successful
	Lobo, et. al. 2018 (REPLENISH trial)	Oral single-capsule estradiol-P4 doses administered daily: 1 mg estradiol + 100 mg P4, 0.5 mg estradiol + 100 mg P4, 0.5 mg estradiol + 50 mg P4, 0.25 mg estradiol + 50 mg P4		Successful
	Moyer, et. al. 1993	1.5 mg or 3 mg / day percutaneous estradiol	200 mg–300 mg OMP variable administration, 10–14 days	Successful
VAGINAL P4	Cicinelli, et. al. 2002	50 mcg / day transdermal estradiol	4% vaginal P4 gel 45 mg 2 applications per week	Mixed Results
	De Ziegler, et. al. 2000	2 mg / day estradiol valerate **OR** 0.625 mg / day CEE **OR** 0.05 mg / day estradiol patch	4% vaginal P4 gel 45 mg 2 applications per week **OR** for 10 days	Successful
	Di Carlo, et. al. 2010	50 mcg / day transdermal estradiol	100 mg–200 mg vaginal P4 days 14-25 of each 28-day cycle	Successful
	Ross, et. al. 1997	0.625 mg / day CEE	45 mg or 90 mg vaginal P4 applications on days 17, 19, 21, 23, 25, and 27 of cycle	Successful
	Sriprasert, et. al. 2021 (ELITE study)	1 mg / day oral micronized 17-beta-estradiol	4% vaginal micronized P4 gel 45 mg for 10 days each month	No
TRANSDERMAL P4	Leonetti, et. al. 2005	0.625 mg / day CEE	2.5 mg medroxyprogesterone acetate (MPA) followed by 20 mg transdermal P4 twice daily or vice versa	Successful
	Vashisht, et. al. 2005	1 mg / day transdermal estradiol	40 mg / day transdermal P4	No
	Wren, et. al. 2000	100 mcg estradiol patch	16 mg, 32 mg, or 64 mg transdermal P4 applications on days 15–28 of cycle	No (even if saliva P4 is high)

Table 1: *Clinical evidence supports the use of oral or vaginal P4 therapies to effectively protect endometrial tissue from hyperplasia. The only study that supports the use of transdermal P4 for endometrial health is by Leonetti and colleagues (2005). This study reports using a synthetic progestin either before or after a course of transdermal P4 therapy. The design of the study inherently makes it impossible to ascertain which therapy (MPA or transdermal P4) is responsible for producing the desired clinical effect of endometrial protection.*

VAGINAL PROGESTERONE

Women who experience side effects with oral P4 therapy may consider vaginal P4 therapy as an alternative, although published evidence assessing its efficacy and optimal regimen is limited in the context of HT. Some studies report that P4 administered vaginally in similar doses (100—200 mg) and duration as with oral therapy, is effective at protecting endometrial tissue from hyperplasia (Table 1) **[16] [17]**.

You may notice that several studies have evaluated doses of 45 mg of vaginal P4, but these studies have mixed results, and we recommend higher dosing (that have demonstrated consistent results) to ensure endometrial protection.

Providers should be careful not to confuse "topical" *vaginal* use of P4 with "topical" transdermal P4 use as the two have very different absorption patterns. Vaginal P4 has been proven to successfully protect the endometrium; transdermal (applied on the skin) P4 has not.

TRANSDERMAL PROGESTERONE & SALIVA TESTING

It is clear from the evidence that transdermal progesterone should not be used to balance ERT. It is also clear that saliva testing, which is often used to affirm the use of TD P4, does not represent tissue uptake of P4 when applied to the skin. See the Appendix for details.

TESTING OPTIONS FOR WOMEN NOT ON P4 THERAPY

Serum testing has been conventionally regarded as the "gold standard" for measuring endogenous sex steroids. This method has the advantage of having well-established reference ranges and a simple sample collection through a blood draw. A significant drawback of serum testing, however, is that it tests a single "snapshot" at a moment in time. Hormones, including P4, are released in a pulsatile pattern throughout the day and night, making it challenging to determine if the serum levels represent a peak, a trough, or something in between.

Salivary P4 measurements are sometimes used to assess endogenous P4 levels, but available technology has mixed reviews. Arslan, et. al., recently showed that the most sensitive and accurate saliva assays, as used in a research environment, demonstrate moderate correlation to serum P4 levels. However, the commercially available saliva assays performed poorly compared to serum. The authors concluded that most commonly used commercial assays exhibit unsatisfactory performance when evaluating salivary P4 **[18]**.

Urine metabolites from the DUTCH Test have demonstrated excellent correlation to serum P4 levels **[19]**. Moreover, urine testing may have an additional advantage in that serum P4 levels can vary significantly due to pulsatile production, while DUTCH averages out hormone production throughout the day by providing a result based upon 4 samples collected over 24 hours rather than a single snapshot.

TESTING OPTIONS FOR WOMEN ON OMP

Serum testing should be avoided in patients on OMP therapy. After taking a dose of OMP, progesterone levels spike rapidly in circulation, maintain their peak concentration for about an hour, and then decline just as rapidly back to baseline. Consequently, it would be nearly impossible to say if a serum test result for P4 in this instance represented a peak,

a trough, or an intermediate state. As serum sampling is typically performed hours after values return to near baseline, adjusting therapy dosing should never be based on P4 levels obtained from serum measurements.

NOTE

Depending on the type of methodology used to assess P4 levels, results obtained from serum analysis may also be erroneously elevated by as much as eightfold due to analytical artifacts from interferences caused by P4 metabolites **[20]**. *LC-MS/MS technology (coupled with serum analysis) could be used to circumvent this issue and improve the specificity of the method.*

Saliva testing should never be used to assess OMP dosing or extrapolate for meaningful clinical impact.

Saliva testing should also be avoided in patients on OMP therapy. As mentioned above, after a dose of OMP, P4 levels rise and fall within hours. This rapid pharmacokinetic profile, combined with the lack of evidence supporting salivary measurement of P4, renders saliva as a useless measurement to monitor OMP therapy.

The rise in urine metabolites when patients take oral hormones is typically dramatically higher than the increase in serum. More than 90% of orally administered P4 is metabolized during first pass metabolism (through the gut and liver) **[21]**. This creates a pool of urine metabolites that represent first pass metabolites of P4 (and not tissue levels). As it relates to assessing OMP's endometrial effects (and therefore dosing adjustments), urine metabolite evaluation is not useful, nor recommended.

Healthcare providers may plan to use progesterone specifically because of its calming, anxiolytic, and sleep-promoting properties. These effects are primarily mediated by P4 alpha metabolites, like a-pregnanediol. While intestinal bacteria and liver can extensively metabolize oral P4, it is the enterocyte cells of the intestinal wall that are primarily responsible for promoting the 5a metabolism of P4 towards its more potent anxiolytic metabolites **[22]**.

DUTCH provides valuable insights that aid clinicians in evaluating individual patient's inherent metabolic tendencies in relation to P4 metabolism. The production of these anxiolytic metabolites from OMP may be beneficial for certain patients dealing with mood or sleep concerns, while being unfavorable for others experiencing excessive drowsiness or dizziness. Testing may provide biochemical rationale for symptoms associated with treatment.

TESTING WOMEN ON VAGINAL P4

One of the unique features of vaginal P4 is the uterine first-pass effect. Hormone placed in the top third of the vagina undergoes direct transport into the uterus. Cicinelli, et. al., reported that the endometrium to serum ratio of P4 was more than 10x higher with vaginal application compared to P4 injections **[23]**. Because of this phenomenon, no lab tests (serum, saliva, or urine) accurately represent endometrial tissue exposure with vaginal P4 administration. So overall, testing is of limited value for patients taking vaginal P4.

DRIED URINE TESTING FOR COMPREHENSIVE HORMONES (DUTCH)

From a broader perspective, laboratory testing should serve as a platform for ensuring that the desired clinical effects are being achieved. Measuring hormones round the clock over a 24-hr period effectively captures hormone secretion throughout the day and night, giving a more in depth look at hormone patterns for a given patient. To circumvent the hassle of a liquid collection, dried urine testing provides an advantage of a straightforward and easy at-home collection. Coupled with state-of-the art mass spectrometry methodology (LC-MS/MS), DUTCH provides an accurate and reliable way to evaluate many hormones and their metabolites. Peer-reviewed published studies have established serum and dried urine hormone correlations as well as liquid and dried urine equivalency making dried urine a natural choice for testing **[24]**. Results can be used as a guide to personalize treatment decisions to improve symptoms and achieve hormonal balance.

TYPE OF P4 THERAPY	RECOMMENDED DOSAGE	TESTING OPTIONS
NOT on therapy (endogenous P4)	N/A	DUTCH, serum (although serum doesn't capture the "entire picture")
OMP	100 mg—200 mg	Testing is not recommended (except to determine metabolism patterns)
Vaginal	100 mg—200 mg	Testing is not recommended
Transdermal (not recommended)	No dose of TD P4 has been proven to be effective for endometrial protection	Testing is not recommended

Table 2: *Progesterone therapy options (alongside estrogen) for endometrial protection include OMP and vaginal P4 (100 mg—200 mg). Testing is not recommended for therapeutic monitoring.*

ORAL MICRONIZED PROGESTERONE FOR ENDOMETRIAL PROTECTION – CONCLUDING THOUGHTS

Although there are several testing options available to monitor endogenous progesterone levels, there are no clinical studies connecting serum or urine values in the context of endometrial protection with progesterone supplementation (Table 2). So, in short, there are no lab tests out there that confirm endometrial protection. Progesterone levels in serum peak within a few hours after taking oral progesterone, but then fall rapidly. So, testing represents a "snapshot" view and does not encompass the entire picture of progesterone and its metabolites that will continue to be bioactive until the next dose. Moreover, levels of progesterone metabolites in urine do not correlate with serum progesterone levels when oral progesterone is used. (Note: this is in contrast to endogenous progesterone levels, a scenario when serum progesterone and urine progesterone metabolite levels do correlate well **[25]**).

In summary, based on published findings, 100—200 mg of OMP offers consistent protection of the endometrium against hyperplasia in women with an intact uterus who are taking concomitant estrogen therapy. Monitoring progesterone therapy with lab testing is not recommended.

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TRANSDERMAL PROGESTERONE, SALIVA TESTING, & THE PROBLEM WITH BOTH

Transdermal Progesterone

P4 preparations are now available in compounded form as a cream or gel, and in theory, may promise good patient compliance when applied to the skin. Research shows, however, that skin penetration of transdermal P4 is variable and fails to ensure adequate circulating levels of this hormone **[26] [27]**. Published clinical findings strongly argue against combination therapy of estrogen with transdermal P4 because it does not adequately safeguard the endometrium from hyperplasia **[28]**.

Of note, a single study in 2005 suggested that transdermal P4 may elicit protective effects on endometrial tissue **[29]**. This study included 26 postmenopausal women who completed the trial and provided evaluable data. These women had already been treated with CEE combined with MPA – which they paused for 2 weeks prior to being randomized to a group of either CEE with MPA or CEE with transdermal P4 for 6 months, at the end of which, the treatment was paused for 2 weeks and switched to the other group's regimen. Endometrial biopsies were performed in the beginning of the study, after the 6 months of treatment, and at the end of the study. Proliferative endometrium was found in 5 out of 26 women (19%) after the combination of CEE with transdermal P4 (before or after MPA), compared with 7 women (27%) after CEE combination with MPA.

NOTE

> *Clinical findings from peer-reviewed, well-designed published studies agree that* **TRANSDERMAL P4 IS NOT AN EFFECTIVE ALTERNATIVE TO OMP** *when it comes to ensuring endometrial health in postmenopausal patients on estrogen therapy.*

The results of this study have yet to be replicated, and similar studies consistently report conflicting results (showing lack of endometrial protection) **[30]**. To date, no other study (including larger, longer studies) reports that transdermal P4 offers adequate endometrial protection with estrogen HT **[31]**! Unfortunately, the 2005 study by Leonetti and colleagues is often erroneously cited to provide evidence that P4, applied to the skin, produces an impactful clinical response, misleading the reader about safety and efficacy of this type of treatment.

THERE IS NO CLINICAL EVIDENCE TO SUPPORT THE USE OF TRANSDERMAL P4 BY ITSELF FOR ENDOMETRIAL PROTECTION.

1. Leonetti, et. al. (2005) is the single study to ever report that transdermal P4 offers endometrial protection with estrogen HT. However, the treatment regimen in this study also included synthetic progestin (MPA) therapy, either before or after P4 administration. Although endometrial protection was achieved in both groups (MPA before P4 and MPA after P4), it is not possible to say that clinical success can be attributed to P4 therapy alone.
2. Other studies (by Vashisht, et. al. (2005) and Wren, et. al. (2000)) evaluated transdermal P4 with estrogen HT (without synthetic progestins), for longer periods of time and with more subjects, demonstrated lack of endometrial protection.
3. Studies have shown that P4 applied to the breast penetrates into breast tissue in high

levels. These findings are often inappropriately extrapolated to suggest systemic uptake of P4. However, when applied to the skin, P4 does not appear in circulation.

4. High salivary P4 levels are often said to be representative of systemic tissue uptake, but there are no published clinical findings to support this position.

Transdermal Progesterone Does Not Protect the Endometrium

Additional studies report that the absorption of transdermal P4 through the skin is rather variable and its availability to tissues fluctuates tremendously **[32]**. Perhaps failure of transdermal P4 to increase P4 levels in the circulation could be responsible for lack of clinical impact–it does not protect the endometrium against hyperplasia **[33]**. The abundance of published clinical evidence provides alignment on the use of transdermal P4 in HT–it is not an effective treatment strategy to ensure endometrial protection against the proliferative impact of estrogen **[34]**.

NOTE

Transdermal P4 does not balance the proliferative effects of estrogen in HT and is therefore associated with an increased risk of developing endometrial hyperplasia and cancer **[35] [36]**.

Saliva is NOT Suitable for Testing Sex Steroids

Curiously, some laboratories continue to advocate for the use of transdermal P4, while erroneously promoting saliva testing as a reliable way to monitor this type of P4 therapy. When applied to the skin, P4 does not produce a dose-dependent response in circulation or in saliva– so there is no scientifically sound way to assess the safety and efficacy profiles of transdermal P4 in protecting the endometrium with HT.

Saliva testing yields highly variable, dramatically elevated, and overall unreliable values that do not correspond to a clinically meaningful biological response in patients **[37]**. Here's the step-by-step explanation of how saliva sampling produces profoundly increased and simultaneously misleading results:

- In the luteal phase, the ovaries produce 20—30 mg of P4 daily (in contrast to <1 mg after the onset of menopause).
- In the luteal phase, P4 levels vary between 70—270 pg/mL in saliva (according to one hormone testing laboratory).
- However, postmenopausal women who use 20—30 mg of transdermal P4 HT to mimic premenopausal physiological production, have saliva P4 levels much higher than the premenopausal luteal range at levels >500 pg/mL 12-24 hours after dosing (according to this same laboratory).
- Peak P4 levels in saliva are typically observed ~2-4 hours after transdermal P4 application; these are reported to be tremendously high at levels >15,000 pg/mL (by the same laboratory), over 50-200 times higher than what is observed in the luteal phase.
- Saliva-testing laboratories postulate that these "on therapy" values are representative of tissue uptake of P4.

It is unclear why transdermal P4 application contributes to such dramatically high P4 values in saliva. What we do know, however, is that hormone creams and gels administered topically increase the levels of the corresponding hormone in saliva to a much higher degree than in serum or urine. At the same time, there is no evidence that these elevated levels correlate to any meaningful clinical results. If saliva levels were truly representative of tissue P4 exposure in response to transdermal P4 administration, we would expect

successful endometrial protection with relatively low doses of P4 cream. But that is not the case **— topically administered P4 does not offer endometrial protection, regardless of elevated saliva levels.**

Vashisht, et. al. reported that P4 cream (40 mg administered daily for 48 weeks) failed to protect the endometrium from proliferative estrogen effects **[38]**. In addition, Wren, et. al., reported clinical failure of P4 cream with regard to endometrial protection (with doses as high as 64 mg) **[39]**. The latter study also included valuable data—saliva levels with topical P4 therapy!

This study showed that women using estrogen therapy and a P4 cream **did not achieve endometrial protection even when saliva results were dramatically elevated.** In other words, the levels of P4 in saliva did not reflect endometrial exposure to this hormone. The authors concluded that the profound variability of salivary P4 deemed this testing modality completely unreliable, and therefore was not of value in managing transdermal P4 therapy.

The "topical/transdermal P4 and saliva testing" theory has not been clinically verified as safe and efficacious, therefore rendering this narrative misleading at best and unsafe for the health of postmenopausal patients at worst. It has perpetuated a paradigm of confusion and discord, with very little focus on the safety and efficacy of clinical solutions in menopause **[40]**.

Frankly, lack of published literature to support topical P4 therapy and salivary testing combination is deeply troubling—without evidence-based findings, how can the dosage for progesterone therapy be optimized to prevent endometrial hyperplasia and prospective malignancy?

Overall, the propensity of saliva sampling to generate inconsistently high results coupled with insufficient evidence for transdermal P4 being utilized in HT to oppose estrogen does not meet the clinical objectives of HT! Providers must insist that well-designed clinical trials that assess safety and efficacy of transdermal P4 together with salivary testing be conducted prior to implementing this approach in their clinical practice.

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