

Endometriosis and the DUTCH Test:

The Relationship between Estrogen, Progesterone, and Cortisol Dysregulation



emerson[®]
ecologics

dutchtest[®]
BY PRECISION ANALYTICAL INC.



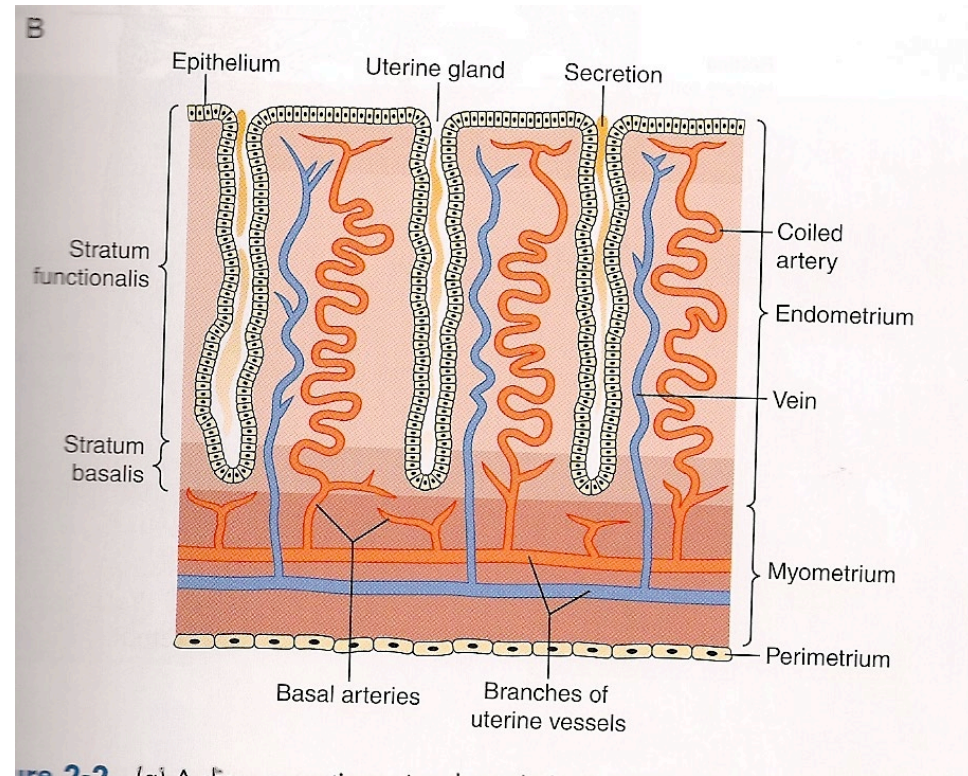
Meet the endometrium!

- Estrogen and progesterone induce functional changes in the endometrium to prepare for implantation and the establishment of pregnancy
- During the luteal phase, the proliferative endometrium becomes secretory
 - Thick, spongy, full of spiral arterioles
 - Glandular epithelium secretes chemokines, growth factors, and cell-adhesion molecules (CAMs)
- These function to create an environment where the blastocyst can implant and the chemokines actually attract the blastocyst to specific sites
- Endometrial epithelium creates dome-like structures called pinopodes which act as “docking sites”
 - Between pinopodes, MUC-1 expression repels embryos



Endometriosis

- Prevalence about 10% in reproductive-age women
- Associated with pain and infertility, and the 3rd most common indication for hysterectomy in women in the US
- Estimates of health care costs from endometriosis in the US in 2002 were \$22 million



About Endometriosis

Characterized by growth of hormone-responsive endometrial tissue outside of the uterine cavity

- Most commonly found on peritoneal surface, within ovary (chocolate cysts), or rectovaginal septum
- More widely distributed lesions have been described
include patient with lesions on diaphragm





Theories of Endometriosis

- Symptoms first described in Dutch and Belgian writings from the 1600s
- First US description was by John A Sampson from Johns Hopkins. He described that lesions arose from endometrial cells which escaped through fallopian tubes during menstruation.
 - Retrograde menstruation and implantation
 - Leading theory still stands





Theories of Endometriosis

- Retrograde menstruation
 - Endometrial cells shed and move to peritoneal and subperitoneal surfaces
 - Express extracellular adhesion molecules
- Coelomic metaplasia
 - Endometrial and peritoneal cells both arise from the same embryologic precursor: the coelomic cell
 - More difficult to prove and support scientifically, but plausible



Retrograde Menstruation- is this the problem?

- Endometrial cells shed and move to peritoneal and subperitoneal surfaces
- Express extracellular adhesion molecules
- Some studies have shown that rates of retrograde menstruation (based upon RBCs in abdominal peritoneal cavity) are similar in women with and without endometriosis
- Only PART of the story!





Risk Factors

- Typically, endometriosis is only active after menarche and before menopause
- Most common in women with early menarche and who experience fewer than 2 pregnancies.
- Also associated with menstrual cycles >30 d
- More prevalent in women with IUD use >2 years
- Increased rates in women who have experienced repeated vaginal and uterine infections.
- Familial relationship
- OC use has mixed data: 1993 review showed nonsignificant risk reduction of 20%. Case control data is mixed.





Known Factors in Etiology

- Estrogen dependence & detoxification pathways
- Environmental exposures
- Polygenic genetic associations
- Inflammation
- Microbiome





Known Factors: Estrogen Dominance

- Estrogen dependance/dominance
 - Concentrations of estrogen >50 pg/mL are needed to support growth
- Environmental exposures
 - Increased odds ratios for endometriosis in women exposed to PCBs and dioxins
 - In-utero exposure to PCBs was shown to induce an endometriosis-like phenotype (decreased progesterone receptors) in females of F1 generation





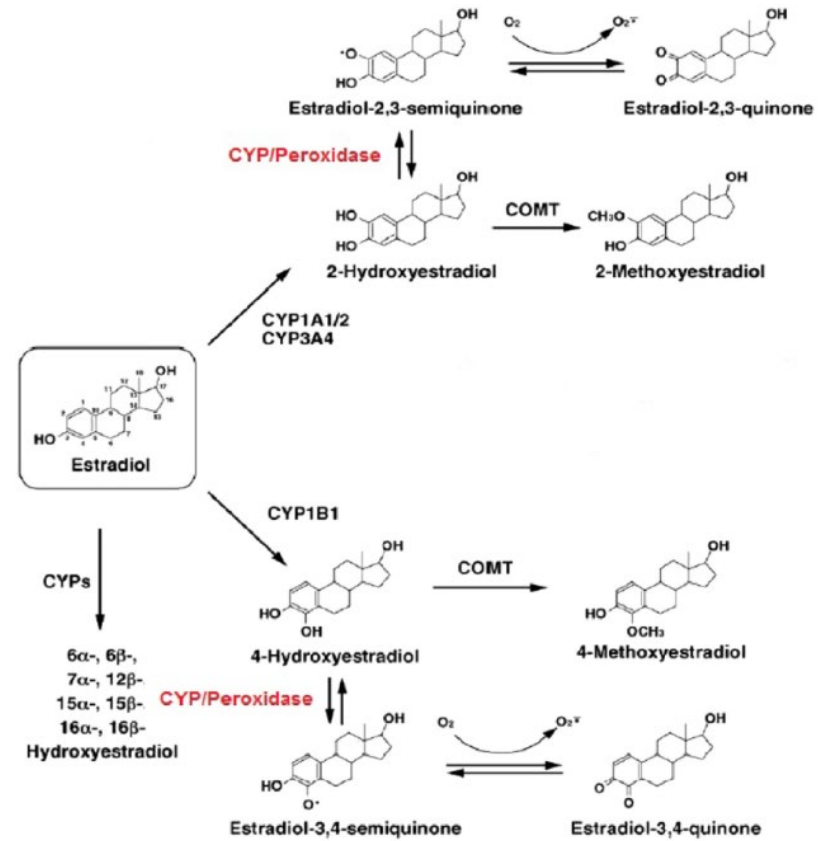
Estrogen & its Detoxification

- 3 key phases of detoxification
- Involves proper functioning of ovaries, liver, stomach, gall bladder, intestines, kidneys and more!



Phase 1 detoxification: Cytochrome Enzyme Systems

- Occurs mostly in the liver
- Estradiol is metabolized using CYP 1A2, 1B1, and 3A4 enzymes, depend on which is most available
- Estrogen undergoes the addition of a hydroxyl group (-OH)
- Highly reactive oxidative intermediates which need further transformation to be excreted
- Metabolites can still act on E receptors (weakly)
- Requires iron, good liver function, alcohol/meds can impede



Phase 1 detoxification: Cytochrome Enzyme Systems

“2-OH” 2-hydroxyestrone & 2-hydroxyestradiol	“4-OH” 4-hydroxyestrone & 4-hydroxyestradiol	“16-OH” 16-hydroxyestrone
CYP1A1 & CYP1A2	CYP1B1	CYP3A4
Less carcinogenic and generally “preferred”	More carcinogenic	Proliferative Can be good for bones, but not so good with breast/fibroids/endo
Weakest binding potential to E receptor Anti-proliferative effects on cancer cell lines	If not properly metabolized, it can turn down a different pathway to become the free radical 3,4-quinone which can cause DNA damage	Binds most strongly to estrogen receptor, though still weakly

All of these metabolites are highly reactive oxidative intermediates- they require further biotransformation!



Phase 2 detoxification: Methylation, Sulphation, Glucuronidation

- 2-OH & 4-OH catechol estrogens get a methyl group added by catechol-O-methyltransferase (COMT)
- Leads to production of
 - 2- and 4-methoxyestrone
 - 2- and 4-methoxyestradiol
- Conjugation with glucuronic acid and sulfate in liver → more water soluble
- Sulphation & glucuronidation to support excretion thru bile into gut (like a package now, all tied up!)



Phase 3 detoxification: Out of cells & into (& out of) gut

- Estrogen metabolites leave the liver thru bile and are excreted through feces or urine
- B-glucuronidase
 - Found in tissues (i.e. breast) but also made in the gut by microbiome
 - When made in the gut, estrogens can be recirculated rather than excreted, increasing estrogen load



Summary

	Phase 1: CYP Enzyme detox	Phase 2: Neutralization & water solubility	Phase 3: Excretion
Key steps	CYP enzymes convert estradiol into 3 main metabolites, 2-OH, 4-OH, 16-OH (which are oxidative!)	Neutralization of metabolites to avoid DNA damage and make more water-soluble	Excretion out of liver cells and into bile to feces/urine (hopefully no excess B-glucuronidase and no excess reabsorption!)
Potential signs of a problem	Excess relative production of 4-OH or 16-OH metabolites	Poor methylation – see lower 2-Methoxy than 2-OH-E1 Anxiety (can also be linked to slow COMT)	BM infrequent Signs of estrogen dominance
How to help	Ensure liver function Requires Iron +DIM, I3C, quercetin, sulforaphane	+sulforaphane (if from food, requires stomach acid!) Requires SAME, choline, Mg, TMG/betaine, methyl B12/folate/Bs, methionine, Zinc INHIBITED BY: E dominance, gut infxn, quercetin, green tea, PCBs, BPA, heavy metals, nutr deficiency	Improve the microbiome BM daily Ensure stomach acid Increase water, fiber + Prebiotics, Calcium D glucarate Unpeeled, raw carrots AVOID: antibiotics, junk food, alcohol

Known Factors in Etiology: Genetics

- 51% of variation in susceptibility thought to be due to genetics
- Genes involved in xenobiotic metabolism, inflammatory response, and steroid action
 - WNT4 gene: each copy of a T at rs2235529 is associated with 1.3 times higher odds of endometriosis
 - CHD5 gene: each copy of a G is associated with 1.4 times lower times odds of endometriosis
 - NFE2L3 gene: each copy of a G at rs12700667 is associated with 0.84 times odds of endometriosis
 - In Asian women, GG genotype at rs9340799 had 10.3 times the odds of endometriosis

Albertsen HM et al. (2013) . "Genome-wide association study link novel loci to endometriosis." *PLoS One* 8(3):e58257.

Falconer H et al. (2012) . "Analysis of common variations in tumor-suppressor genes on chr1p36 among Caucasian women with endometriosis." *Gynecol Oncol* 127(2):398-402.

Painter JN et al. (2010) . "Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis." *Nat Genet*.

Hsieh YY et al. (2007) . "Estrogen receptor alpha-351 XbaI*G and -397 PvuII*C-related genotypes and alleles are associated with higher susceptibilities of endometriosis and leiomyoma." *Mol Hum Reprod* 13(2):117-22.





Known Factors: Inflammation

- Inflammation and endometriosis
 - In early endometriosis, elevated levels of cytokines, lymphocytes, and prostaglandins in peritoneal fluid
 - Decreased T-cell reactivity and NK cell cytotoxicity, which leads to increased likelihood of implantation of endometriotic tissue
 - Some autoimmune antibodies have also been seen in early endometriosis





Known Factors: Microbiome & GI Health

- A healthy gut minimizes reabsorption of estrogen from the gut allowing safe removal in stool and urine
- Inflammation also linked to GI health & microbiome
- Endometriosis appears to be associated with an increased presence of Proteobacteria, Enterobacteriaceae, Streptococcus spp. and Escherichia coli across various microbiome sites.
- The phylum Firmicutes and the genus Gardnerella also appear to have an association (vaginal)
- Consider calcium D-glucarate to inhibit B-glucuronidase





Known Factors: Microbiome & GI Health

- Improve the microbiome with probiotics, prebiotics, diverse diet!
- At least one BM daily
- Increase water & fiber intake (Unpeeled, raw carrots)
- Calcium D-glucarate
- Movement!
- **AVOID:** antibiotics, junk food, alcohol



“Enriched Environment” & Stress as Exacerbating Factor

2018 rat study where rats were exposed to either “no enrichment (NE)” or “enriched environment (EE)” starting on postnatal day 21 x 8 weeks, then surgically implanted with endometriosis

- EE- more space, toys, nesting materials, more socialization

After 60 days, they were reassessed

The EE group showed 28% reduction in endometriosis vesicles and vesicles were significantly smaller

EE group had lower baseline anxiety behaviors, lower CRH (corticotropin releasing hormone) and GR (glucocorticoid receptors) in vesicles

Stress reduction can play an important role in

We know that stress can drive further development of endometriosis

Torres-Reverón A, Rivera LL, Flores I, Appleyard CB. Environmental Manipulations as an Effective Alternative Treatment to Reduce Endometriosis Progression. *Reprod Sci.* 2018 Sep;25(9):1336-1348. PMID: 29137551





How can the DUTCH test help?

- Understand more about estrogen
- Identify markers of inflammation and oxidative stress
- HPA axis function evaluation





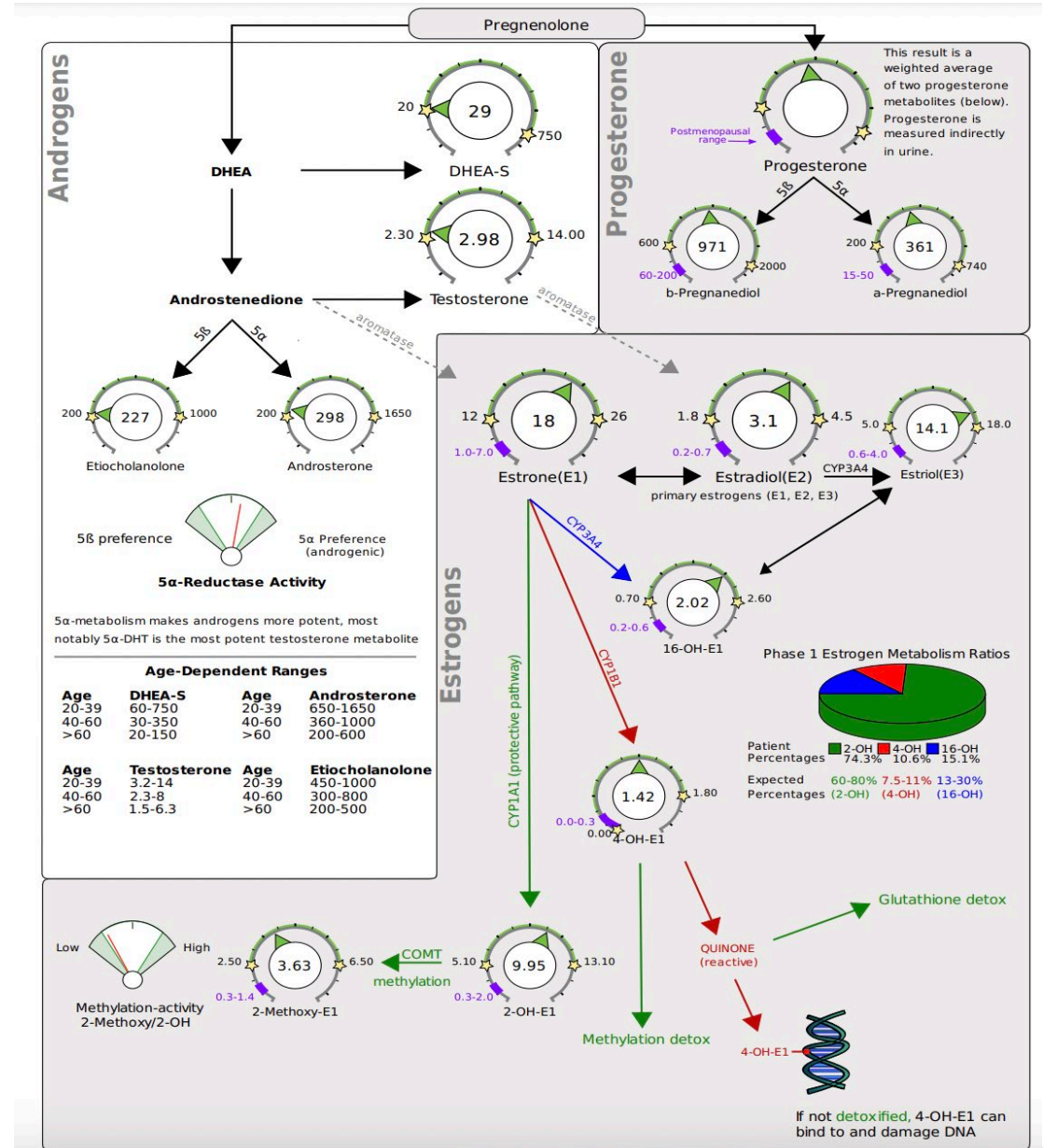
Estrogen and it's role

- Estrogen and some of its metabolites are known to drive proliferation of endometriosis (and other tissues- some good, like bone!)



Understanding Estrogen

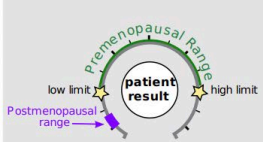
- DUTCH Plus p3
- Look at total E1, E2, E3
- Relative progesterone
- Phase 1 detoxification
- Phase 2- COMT activity



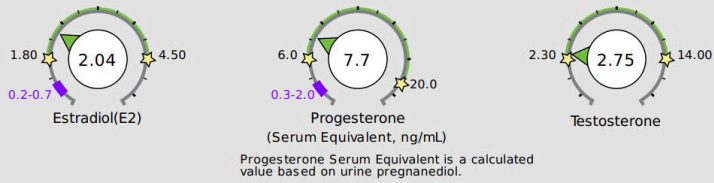
Understanding Estrogen

Hormone Testing Summary

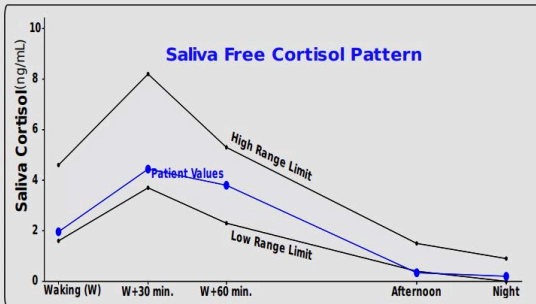
Key (how to read the results):



Sex Hormones See Pages 2 and 3 for a thorough breakdown of sex hormone metabolites



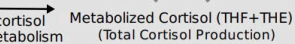
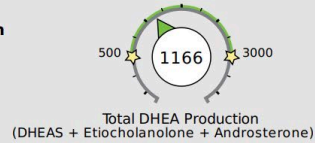
Adrenal Hormones See pages 4 and 5 for a more complete breakdown of adrenal hormones



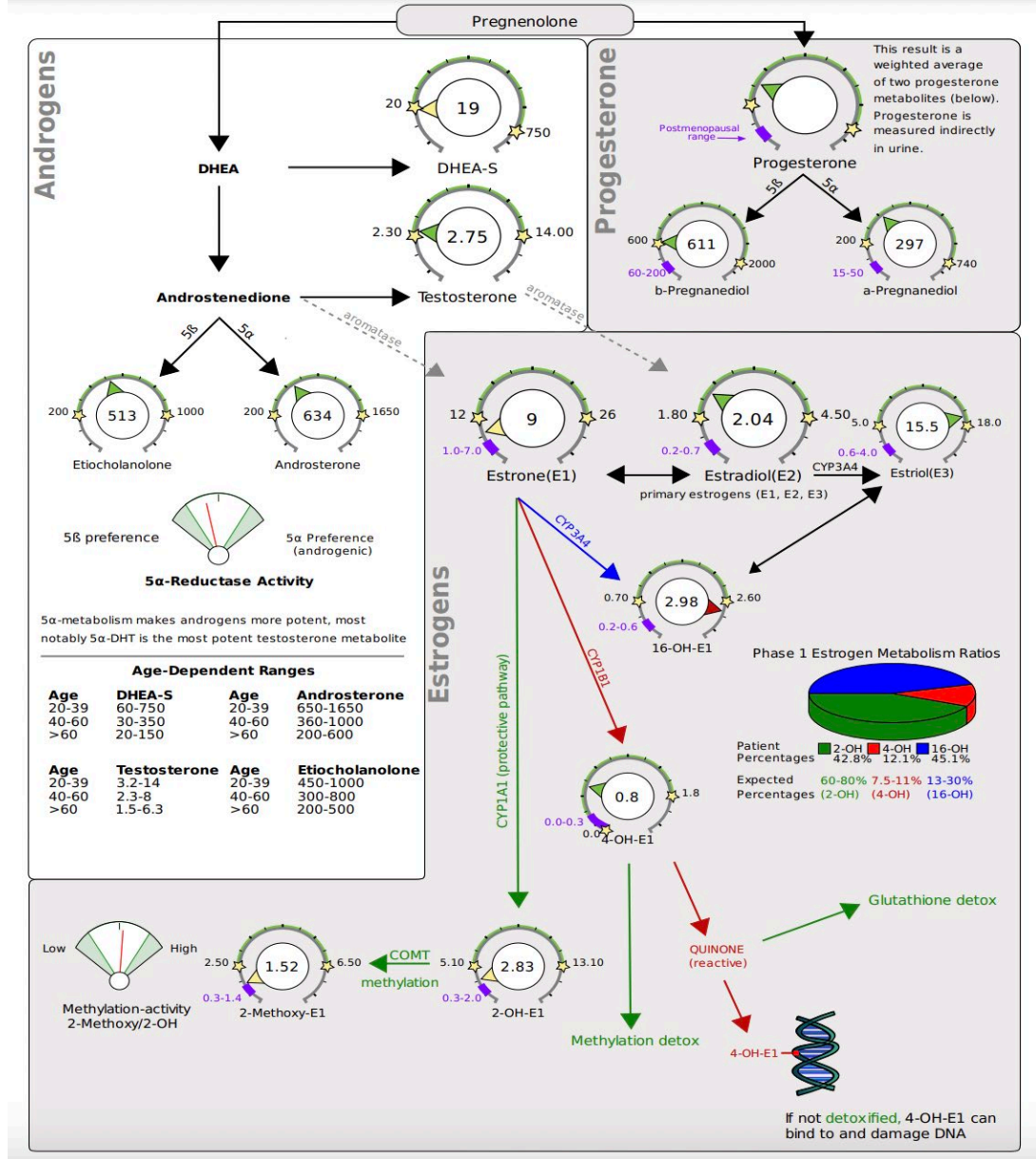
Free cortisol best reflects tissue levels. Metabolized cortisol best reflects total cortisol production.

Total DHEA Production

Age	Range
20-39	1300-3000
40-59	750-2000
>60	500-1200



cortisol metabolism



How can the DUTCH Test help?

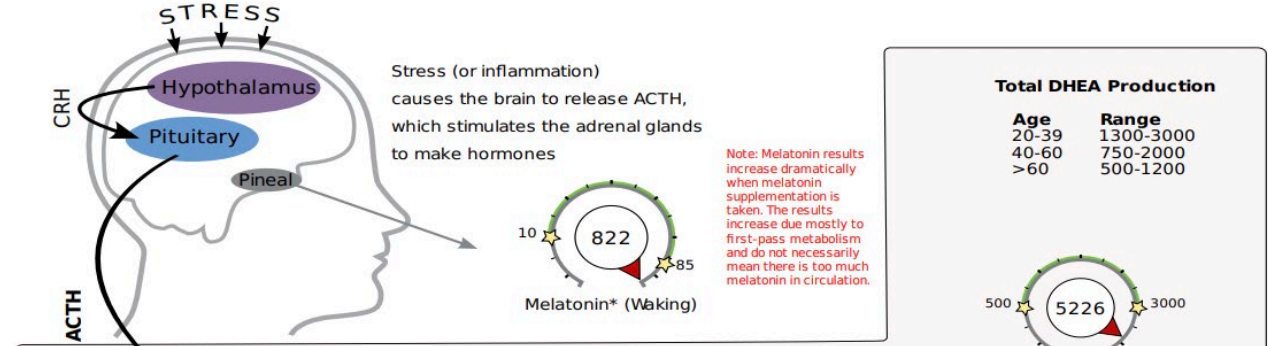
- Identify markers of inflammation and oxidative stress & underlying deficiencies/drivers

Category	Test	Result	Units	Normal Range
Nutritional Organic Acids				
Vitamin B12 Marker (may be deficient if high) - (Urine)				
	Methylmalonate (MMA)	Within range	1.3 ug/mg	0 - 2.5
Vitamin B6 Markers (may be deficient if high) - (Urine)				
	Xanthurenate	Within range	0.78 ug/mg	0.12 - 1.2
	Kynurenate	Within range	2.6 ug/mg	0.8 - 4.5
Glutathione Marker (may be deficient if low or high) - (Urine)				
	Pyroglutamate	Within range	44.5 ug/mg	28 - 58
Neurotransmitter Metabolites				
Dopamine Metabolite - (Urine)				
	Homovanillate (HVA)	Within range	8.7 ug/mg	3 - 11
Norepinephrine/Epinephrine Metabolite - (Urine)				
	Vanilmandelate (VMA)	Within range	3.6 ug/mg	2.2 - 5.5
Melatonin (*measured as 6-OH-Melatonin-Sulfate) - (Urine)				
	Melatonin* (Waking)	Low end of range	15.3 ng/mg	10 - 85
Oxidative Stress / DNA Damage, measured as 8-Hydroxy-2-deoxyguanosine (8-OHdG) - (Urine)				
	8-OHdG (Waking)	Within range	2.6 ng/mg	0 - 5.2



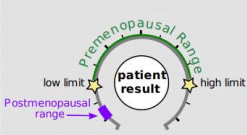
How can the DUTCH Test help?

HPA axis function evaluation

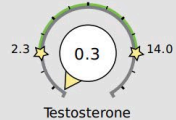
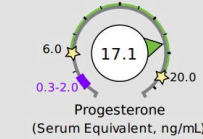
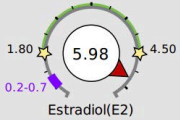


Hormone Testing Summary

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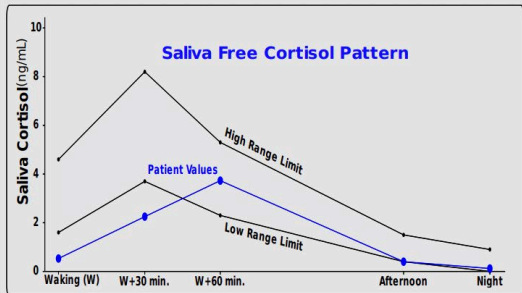


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Progesterone Serum Equivalent is a calculated value based on urine pregnenediol.

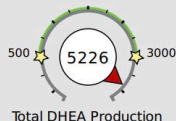
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Free cortisol best reflects tissue levels. Metabolized cortisol best reflects total cortisol production.

Total DHEA Production

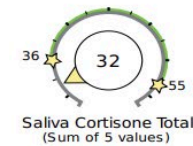
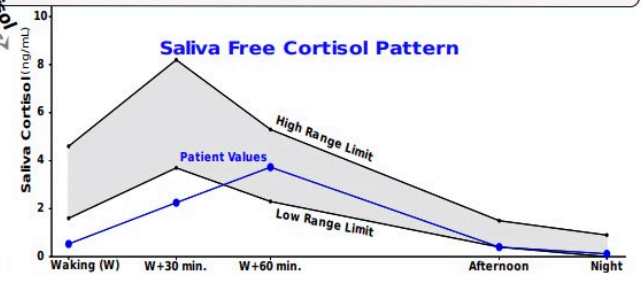
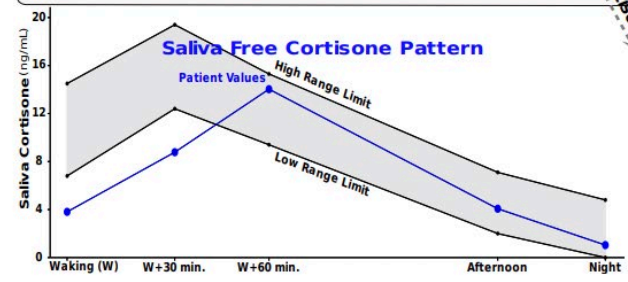
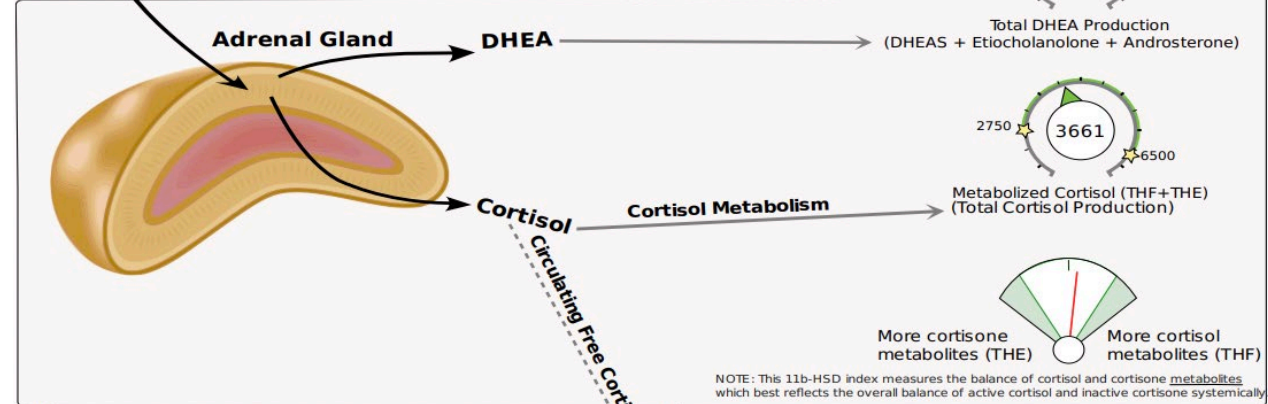
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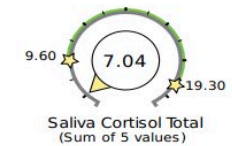
Total DHEA Production (DHEAS + Etiocholanolone + Androsteron)



Saliva Cortisol Total (Sum of 5 values) → cortisol metabolism → Metabolized Cortisol (THF+THE) (Total Cortisol Production)



Cortisol and Cortisone interconvert (11b-HSD)





Endo & Fertility

- Infertile women are 6-8 times more likely to have endometriosis than fertile women.
- Proposed mechanisms include distorted pelvic anatomy, endocrine/ovulatory abnormality, altered peritoneal function, and altered hormonal and cell-mediated functions in the endometrium.
- Ovulatory disorders include
 - Luteinized, unruptured follicle syndrome, impaired folliculogenesis, luteal phase defect, premature or multiple LH surges
- Peritoneal changes associated with inflammation





Conventional Treatment Options

- Mainly pharmaceutical and surgical treatment approaches, depending on staging and severity, symptom picture, and women's goals and needs.
 - I.e. desire to have children, years to menopause, etc.
- Pharma options:
 - NSAIDS for pain management
 - Hormonal therapies
 - Progestins, GnRH analogs, Danazol, OCPs
- Few RCTs, even for conventional treatments.





Naturopathic Treatment Options: Overview

- Understand that endometriosis has multifactorial causes
 - Immune dysregulation
 - Inflammation
 - Hormone dysregulation
 - Diet and Nutritional Status
 - Lifestyle
 - Exposure to exogenous estrogens
 - Stress
- Condition is very difficult to treat!





Naturopathic Treatment Options: Nutrition

- Plant-based diet
- High fiber, low fat
- Green tea
- Decrease intake of alcohol, red meat, dairy, sugar, and caffeine
- Increase dietary use of anti-inflammatory and aromatic spices like turmeric, rosemary, ginger, milk thistle, burdock, and flaxseed
- Omega-3 intake: aim for 2000 mg EPA + DHA daily
- Also flax, evening primrose, borage oils
- Vitamin D Deficiency is associated with endometriosis- supplement it!
- Go organic
- Avoid storage in plastics- stick with glass/stainless





Naturopathic Treatment Options: Pain Relief

- Pain relief
 - Consider botanicals that are analgesic, anodynes, sedatives, antispasmodic, and/or anti-inflammatory
 - Analgesic/anodyne: Dong Quai
 - Uterine sedative: Black cohosh, California poppy, white peony, kava kava
 - Anti-inflammatory: Dong Quai, calendula, echinacea, green tea, curcumin, evening primrose, ginger
 - Anti-spasmodic: wild yam, black cohosh, white peony, cramp bark, black haw





Naturopathic Treatment Options

Immunomodulation

- Dong Quai
- Astragalus
- Calendula
- Ashwagandha

- Improve uterine circulation
 - Lady's Mantle (*Alchemilla vulgaris*)
 - Ginger
 - Blue Cohosh (*Caulophyllum thalictroides*)
 - Calendula
 - Manual therapies (in PT setting, shown to increase pregnancy rates)





TCM Treatments

- TCM has described endometriosis as a condition of blood stasis with formation of abdominal lumps.
- Underlying causes of blood stasis include
 - qi stagnation (restricted circulation sometimes caused by emotional distress)
 - Coldness (impaired metabolism and circulation) sometimes described as kidney yang deficiency
- Popular commercially available formulas in the US include
 - Lindera 15 (regulates qi and blood and relieves pain)
 - Lower Palace Tablets (based on Shaofu Zhuyu Tang)
 - Turtle Shell Tablets (based on Neiyi Wan #1)





TCM Treatments

TCM literature is dominated by the application of the component Gossypol, from cotton root and seed.

- Effect like GnRH drug
- Antagonizes action of estrogen and progesterone
- Can cause sx like menopause with continued use, including amenorrhea, atrophy of endometrium
- Studies show short-term effectiveness close to 90% and long-term (1-3 years) maintained in 54-63% of treated.
 - After treatment for several months, amenorrhea persists about 6 months in 80% of women treated, and up to a year in 16% of women treated.
- Also used for cysts and fibroids
- SE include hypokalemia (promotes K secretion), can increase liver enzymes in high dosage
- Gossypol acetate 20 mg plus 10% potassium citrate (10 ml TID) (Drug in China)
- Cotton root has been used in SE US traditionally as well



Case: 31 yo female with unexplained infertility

- TTC x 1 year
- Regular, 26d cycle. Bleeds x 5d
- OB suspects endometriosis-
 - Heavy bleeding with clots
 - Severe cramping cd 1-2
 - Sister struggled with infertility due to diagnosed endometriosis
 - HSG showed 1 fallopian tube blocked, presumed endometrial implant
- Very clean diet & lifestyle profile
- Partner worked up and wnl



Lab Results- Conventional

Cycle Day 3

- Estradiol 58.9 (19.5 - 50 pg/mL)
- FSH 5.23 (3.85-8.78 mIU/mL)
- AMH 2.04 (1.03-11.1 ng/mL for women 26-30)

Cycle Day 24

- Progesterone 15.79 (5.1-18.5 ng/mL)
- TSH 1.6 (0.5-2.0 mIU/mL for fertility)
- Testosterone, total 24 (8-48 ng/dL)
- Testosterone, free 2 (1-5 pg/mL)
- DHEA sulfate 234 (45-270 ug/dL)
- Low normal hemoglobin and hematocrit



Lab Results- Conventional

Cycle Day 3

- Estradiol 58.9 (19.5 - 50 pg/mL) ← Elevated slightly
- FSH 5.23 (3.85-8.78 mIU/mL)
- AMH 2.04 (1.03-11.1 ng/mL for women 26-30)

Cycle Day 24

- Progesterone 15.79 (5.1-18.5 ng/mL) ← Quite good!
- TSH 1.6 (0.5-2.0 mIU/mL for fertility)
- Testosterone, total 24 (8-48 ng/dL)
- Testosterone, free 2 (1-5 pg/mL)
- DHEA sulfate 234 (45-270 ug/dL)
- Low normal hemoglobin and hematocrit



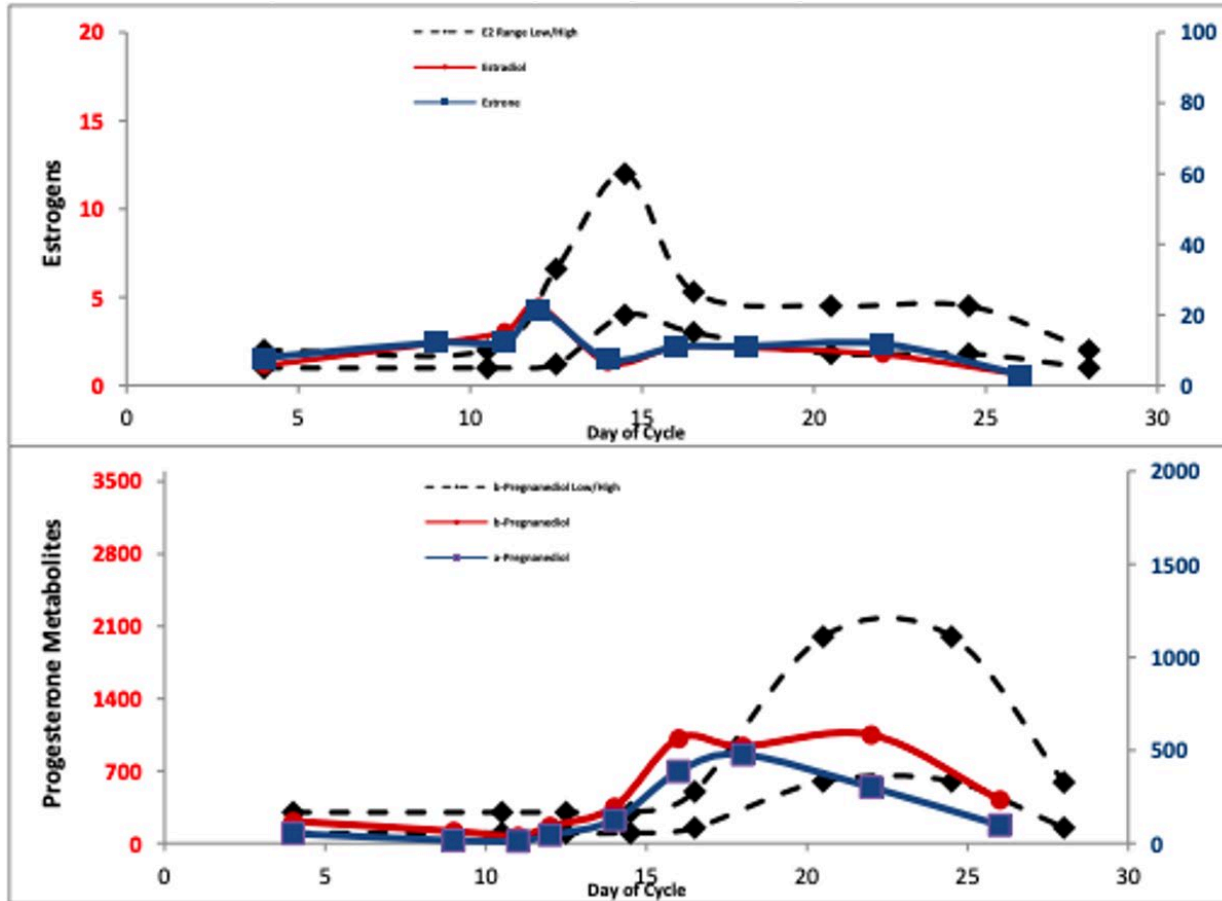
Next Visit

- Her first plan focused on improving estrogen metabolism, optimizing egg health, lengthening her cycles
- Her cycles changed for the worse (shortened) on this plan, and we had to dig deeper
- Ordered a cycle map, measuring hormones throughout the cycle as well as looking at hormone metabolites (she didn't want to do this at first due to out-of-pocket cost)



DUTCH - Cycle Mapping

Monthly Pattern of Urinary Estrogen and Progesterone Metabolites



Measurement	1	2	3	4	5	6	7	8	9
Day of Cycle	4	9	11	12	14	16	18	22	26
Estadiol (E2)	1.1	2.4	3.1	4.5	1.3	2.2	2.1	1.7	0.6
Estrone (E1)	7.9	12.1	12.1	21.2	7.5	10.9	11.1	11.8	3.1
a-Pregnanediol	52	15	12	44	125	386	476	301	97
b-Pregnanediol	213	117	68	165	351	1014	939	1049	422
b-Pg/E2 Ratio	190	49	22	37	274	463	440	601	684

Normal Ranges

Estradiol
Estrone
a-Pregnanediol
b-Pregnanediol
b-Pg/E2 Ratio

Follicular

1-2ng/mg
4-12ng/mg
25-100ng/mg
100-300ng/mg
50-300

Ovulatory

4-12ng/mg
22-68ng/mg
25-100ng/mg
100-300ng/mg
<100

Luteal

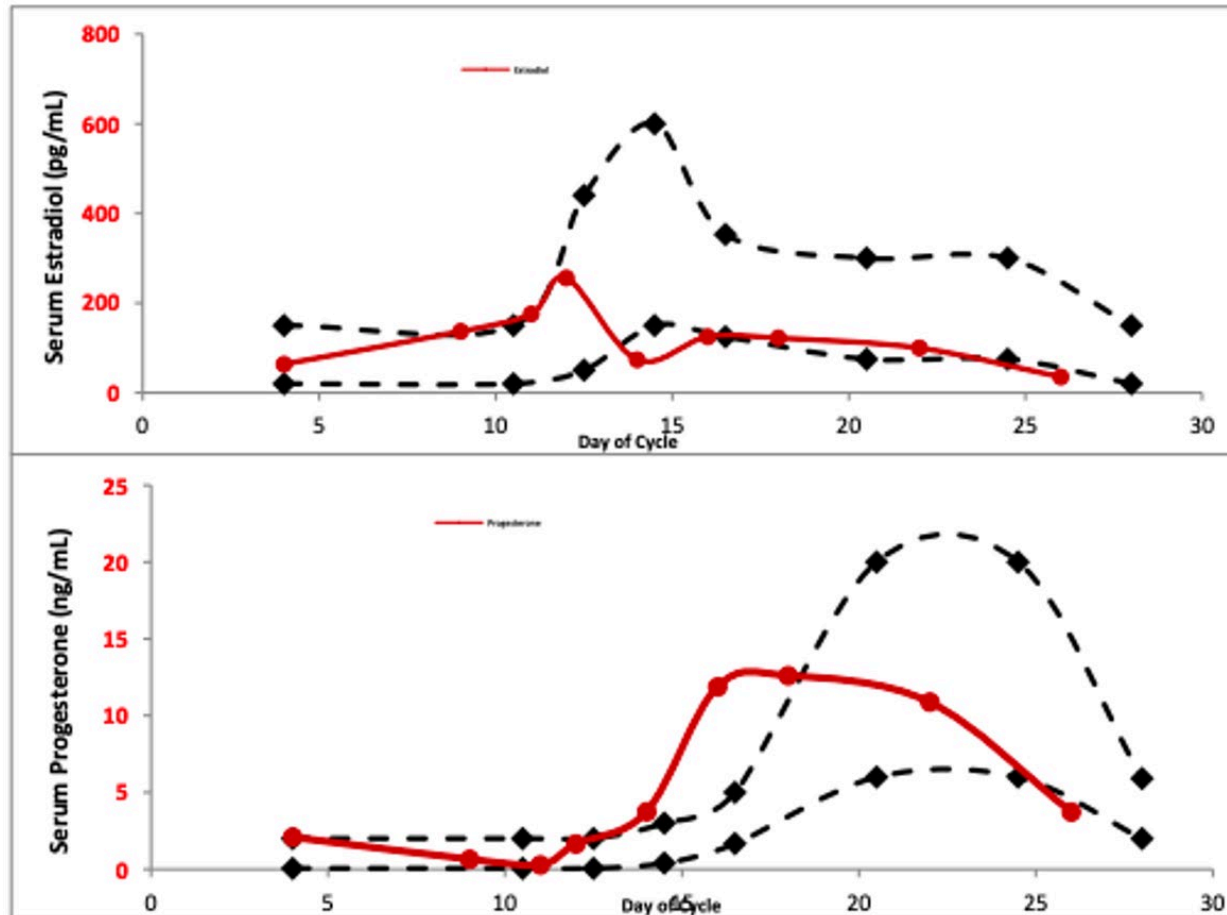
1.8-4.5ng/mg
12-26ng/mg
200-740ng/mg
600-2000ng/mg
100-500

Postmenopausal

0.2-0.7ng/mg
3.0-7.0ng/mg
15-50ng/mg
60-200ng/mg
50-300

DUTCH - Cycle Mapping

Monthly Pattern of Estradiol and Progesterone - Serum Equivalent Estimates*



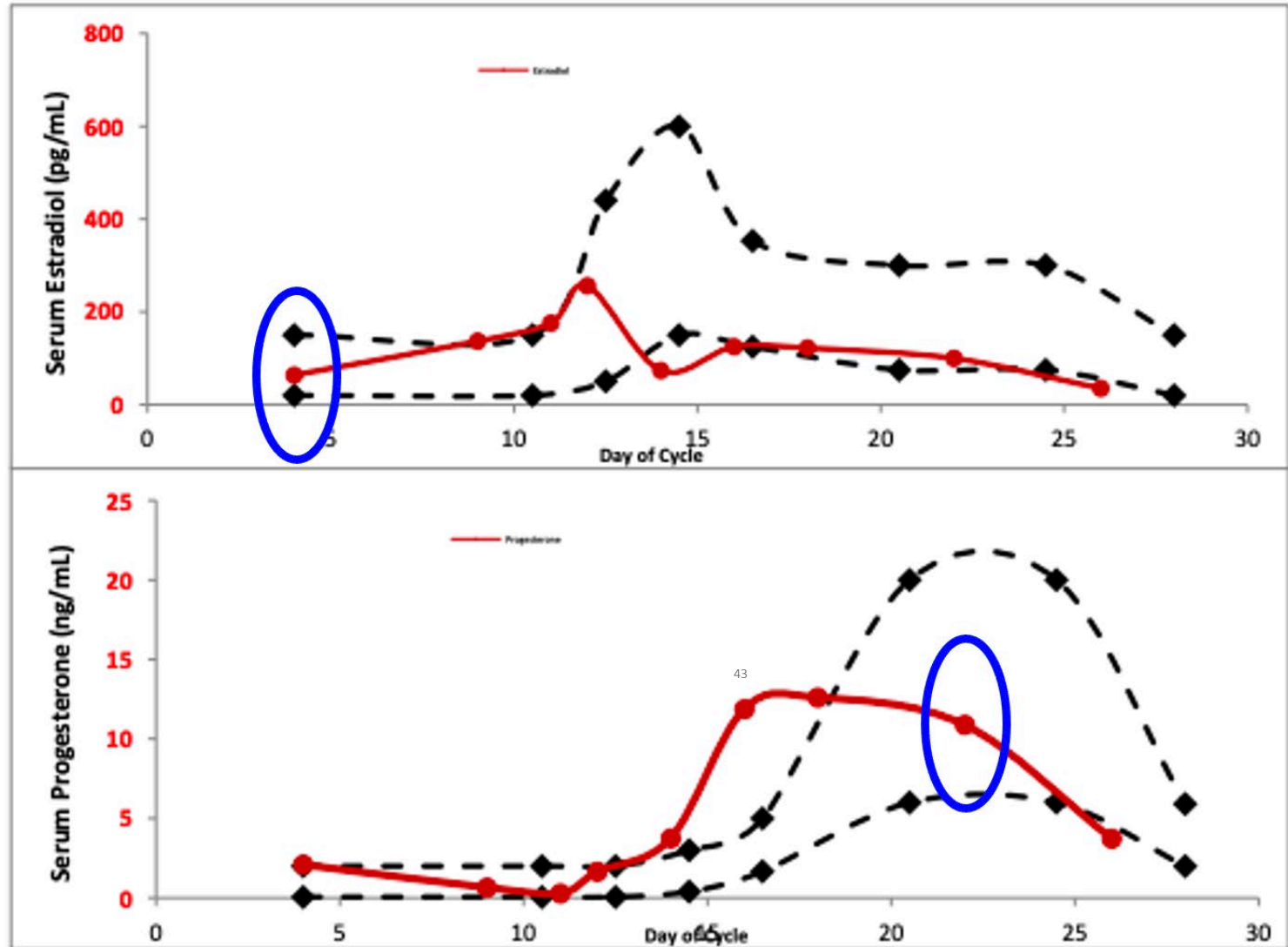
Measurement	1	2	3	4	5	6	7	8	9
Day of Cycle	4	9	11	12	14	16	18	22	26
Estradiol (pg/mL)	63.8	136.6	175.3	256.3	73.2	125.0	121.9	99.6	35.2
Progesterone (ng/mL)	2.1	0.7	0.3	1.7	3.8	11.9	12.6	10.9	3.7

*Values given on this page are based on research in which women's serum (estradiol and progesterone) and urine metabolites (estradiol and a/b-pregnanediol) were both measured. Actual serum measurements were not made for this patient. These values are "research only" and are for informational purposes. The "serum equivalent" numbers are plotted with a luteal reference range of 75-300pg/mL for estradiol and 6-20ng/mL for progesterone based on serum lab values for healthy, premenopausal women. While the correlation between this test and blood levels is excellent, actual serum values may differ somewhat if measured.

DUTCH - Cycle Mapping

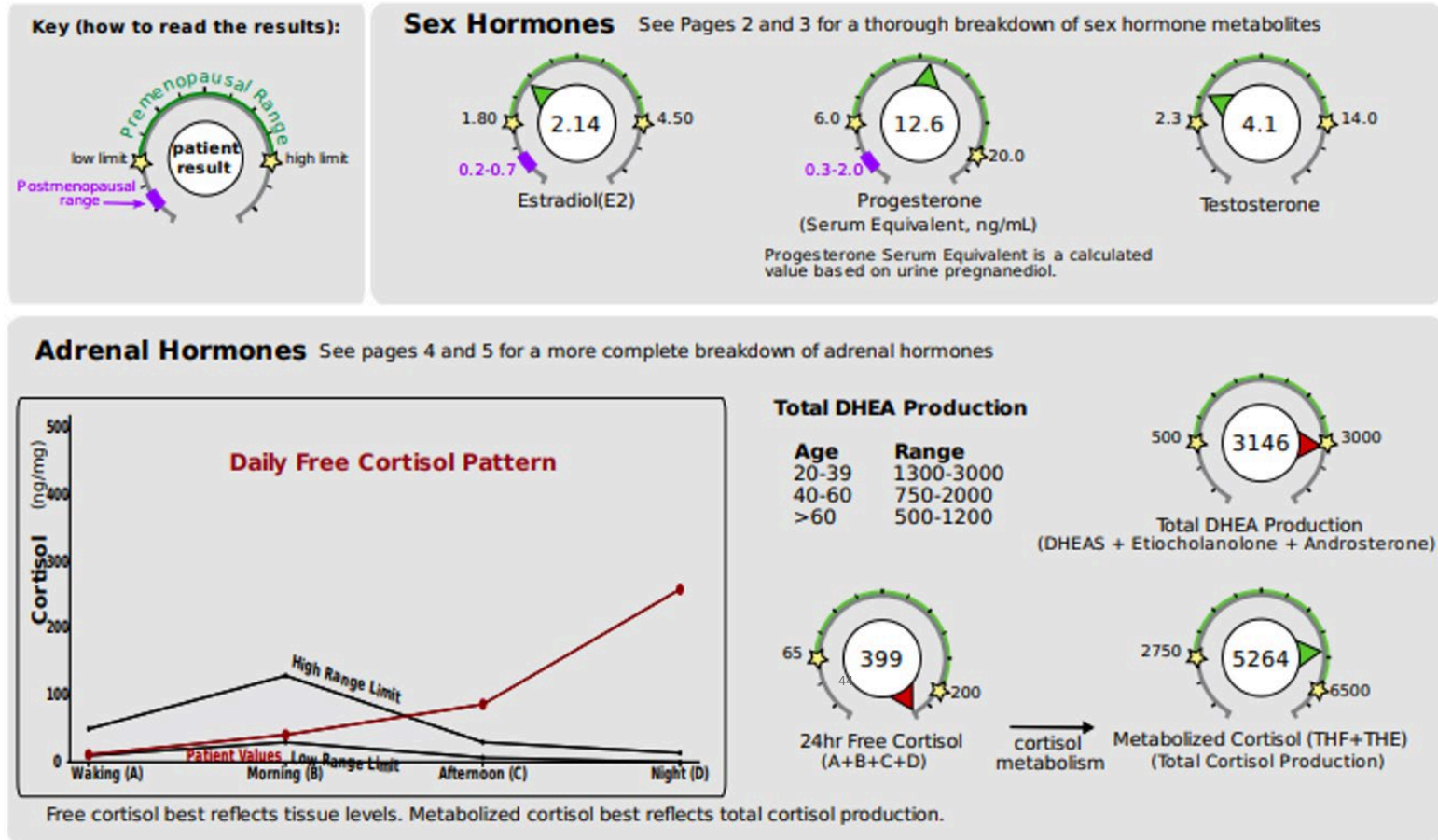
Monthly Pattern of Estradiol and Progesterone - Serum Equivalent Estimates*

Her levels of estrogen and progesterone look normal pretty much ONLY on the days we tested in serum!



Hormone Testing Summary

- Her estrogens were actually quite LOW!
- Cortisol pattern irregular
- She does have metabolic preference to 4OHE1 and 2OHE1 which is the most common pattern in endometriosis
- Inefficient conversion of 2OHE1 to 1methoxyE1 → low methylation activity



The following videos (which can also be found on the website under the listed names along with others) may aid your understanding:
[DUTCH Complete Overview](#) [Estrogen Tutorial](#) [Female Androgen Tutorial](#) [Cortisol Tutorial](#)

PLEASE BE SURE TO READ BELOW FOR ANY SPECIFIC LAB COMMENTS. More detailed comments can be found on page 7.

Adjusted Her Plan

- DIM assists in directing estrogen down the 2-OH phase 1 estrogen detox pathway and away from the 4-OH (which is more problematic)-- GOOD!
- But it also lowers total estrogen in circulation -- NOT GOOD!
- And it stresses her slow COMT phase 2 detox pathway-- NOT GOOD!
- We need to focus more on COMT support to balance her estrogen metabolism to be more favorable, and ensure we do not reduce circulating estrogens!!
 - Mg, Zn, SAM-E, TMG, choline, methyl Bs, methionine, microbiome (to ensure elimination is efficient)



- On her new plan, including COMT support, herbal tinctures to support hormone balance in both phases of her cycle, her cycle lengthened by about 1 day per month.
- About 3 months later, her ovulation (detected by OPK) had pushed out to CD 14 and she had a 28d cycle
- Conceived 2 cycles later

We would have struggled to achieve this without functional lab testing!







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If you'd like to learn more about Integrative Approaches to Infertility,
Please visit Dr. Chasse at

<https://www.perfectfertility.com/ifmwebinar>

Thank you!



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