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Updates on Molecular and Environmental Determinants of Luteal Progesterone Production

Natalie A. DeWitt, Shannon Whirledge, PhD, Amanda N. Kallen, MD*

Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale School of Medicine, New Haven, CT

Abstract

Progesterone, a critical hormone in reproduction, is a key sex steroid in the establishment and maintenance of early pregnancy and serves as an intermediary for synthesis of other steroid hormones. Progesterone production from the corpus luteum is a tightly regulated process which is stimulated and maintained by multiple factors, both systemic and local. Multiple regulatory systems, including classic mediators of gonadotropin stimulation such as the cAMP/PKA pathway and TGF β -mediated signaling pathways, as well as local production of hormonal factors, exist to promote granulosa cell function and physiological fine-tuning of progesterone levels. In this manuscript, we provide an updated narrative review of the known mediators of human luteal progesterone and highlight new observations regarding this important process, focusing on studies published within the last five years. We will also review recent evidence suggesting that this complex system of progesterone production is sensitive to disruption by exogenous environmental chemicals that can mimic or interfere with the activities of endogenous hormones.

Keywords

progesterone; corpus luteum; steroidogenesis; granulosa cell; endocrine disrupting chemicals

Introduction

Scope of review

The ovarian steroid hormones are essential for reproduction but also have important roles in the cardiovascular, central nervous, and skeletal muscle systems. One of the most critical hormones in reproduction, progesterone, is a key sex steroid in the establishment and maintenance of early pregnancy. For example, the synchronous establishment of endometrial receptivity requires the normally compact endometrial stromal cells to decidualize,

*Corresponding Author: Amanda Kallen, MD, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology and Reproductive Sciences, 310 Cedar St, PO Box 208063, New Haven CT 06512, Amanda.kallen@yale.edu, (860) 402-4217.

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transforming into enlarged, secretory cells. This process is regulated by progesterone, where the failure in either the timing or magnitude of decidualization can cause implantation failure and miscarriage^{1,2}. Corpus luteum progesterone production is a tightly regulated affair, which is stimulated and maintained by multiple factors, both systemic and local. Gonadotropins are critical endocrine factors regulating corpus luteum function. However, while gonadotropin-mediated pathways are recognized as broad mediators of steroidogenic signaling pathways, the mechanisms driving granulosa cell (GC) progesterone production are much more intricate than previously recognized, with both local and systemic factors interacting to promote successful GC function and physiological fine-tuning of progesterone levels.

Here, we will highlight new observations regarding mediators of luteal progesterone production, focusing on integrating the various agonists and antagonists of progesterone production into a comprehensive network, when possible. While this review primarily highlights recent findings, many key observations were made prior to this time period; thus, for comprehensive reviews of historic studies, please see ³ and others referenced as follows. Moreover, a growing body of evidence suggests that exogenous compounds known as endocrine disruptors can cause adverse effects on progesterone production, reproduction, and fertility, a topic which has garnered increasing attention (and controversy). Thus, after reviewing recent updates in key signaling pathways regulating progesterone synthesis, we will turn our attention to a review of recent evidence regarding the effect of environmental and endocrine disrupting compounds on progesterone production and discuss areas of potential future investigation which may help clarify these effects.

Production of Progesterone

The two-cell, two-gonadotropin theory and the hypothalamic-pituitary-ovarian axis

The classic “two cell, two gonadotropin theory” establishes that ovarian steroids are synthesized via cooperative interactions between theca and GCs of the ovary⁴. During the follicular phase, androstenedione produced in theca cells diffuses into preovulatory GCs to allow for estrogen synthesis. Serum estradiol levels gradually rise, and pituitary gonadotropin follicle stimulating hormone (FSH) stimulates the formation of luteinizing hormone (LH) receptors on the GCs of the preovulatory follicle⁵. These LH receptors allow for low levels of progesterone to be secreted by the GCs; during the follicular phase, average serum progesterone levels are approximately 0.2 ng/mL⁶.

When follicle maturation is complete, heralded by a dramatic increase in estradiol from the preovulatory follicle, the pituitary LH surge triggers a dramatic shift in the function of the ovarian follicle. At the LH peak, mean serum progesterone levels are approximately 0.8 ng/mL⁶. Approximately 10–12 hours after the LH peak, ovulation occurs, marking the start of the luteal phase, and the remaining GCs in the ovary that are not released by the oocyte differentiate and combine with theca-lutein cells and form the corpus luteum (CL)⁵. These GCs acquire the ability to synthesize tremendous amounts of progesterone⁵ (with average serum levels of approximately 2.3 ng/mL during the early luteal phase)⁶. Approximately eight to nine days after ovulation, in the mid-luteal phase, the CL achieves peak vascularization and progesterone production (with average serum levels of approximately

11.4 ng/mL⁶). During the late luteal phase (10 to 14 days after the LH peak) average serum progesterone levels decrease to approximately 4.4 ng/mL⁶, and if embryo implantation does not occur, the CL degenerates, forming the corpus albicans, and serum progesterone levels fall⁵.

Synthesis of progesterone from cholesterol

Derived from the precursor cholesterol, luteal cells produce progesterone utilizing three cholesterol-modifying enzymes: the steroidogenic acute regulatory protein (STAR), P450 cholesterol side chain cleavage enzyme (P450_{scc}; encoded by *CYP11A1*) and 3 β -hydroxysteroid dehydrogenase (3 β -HSD). The movement of cholesterol from the outer to the inner mitochondrial membrane represents the initial, rate-limiting step in progesterone production and is catalyzed by STAR (reviewed in ^{7,8}). STAR expression in the human ovary follows a temporal pattern. STAR is detectable in theca-interstitial cells during follicular development, while pre-ovulatory GCs synthesize very little STAR⁹. The onset of the LH surge triggers ovulation and GC luteinization, at which time GC STAR expression is dramatically upregulated and GCs gain the ability to produce progesterone in support of a growing pregnancy⁹. STAR is most abundant in post-ovulatory, luteinized follicles, declining significantly toward the end of the luteal phase of the menstrual cycle. This temporal pattern of expression is primarily regulated by circulating gonadotropins, which activate the cyclic AMP/protein kinase A (cAMP/PKA) signaling pathway as well as non-cAMP-driven pathways (reviewed in ¹⁰). Following cholesterol transport from the outer to the inner mitochondrial membrane, P450_{scc} catalyzes the conversion of cholesterol to pregnenolone on the inner mitochondrial membrane. In the ovary, pregnenolone can be further metabolized to 17-hydroxypregnenolone (a precursor to dihydroepiandrosterone) by CYP17, or to progesterone by 3 β -HSD in the smooth endoplasmic reticulum.

Updates on key signaling pathways regulating luteal progesterone

Novel PKA-regulated signaling pathways: beyond gonadotropins

The most well-studied signaling pathway for gonadotropin-stimulated luteal progesterone production is the cAMP/PKA pathway¹¹. It is becoming increasingly clear that the signaling pathways regulated by PKA are more complex than previously realized. PKA activation in isolated rat preluteinized GCs, independent of FSH, is sufficient to induce multiple intracellular signaling pathways, including CREB, β -catenin, and the protein kinase B (AKT) and mitogen-activated protein kinase (MAPK) pathways, and can stimulate transcripts associated with progesterone production including *Lhhcg*, *Star* and *Cyp11a1*¹². The AKT pathway is a known activator of mammalian target of rapamycin (mTOR), a member of the phosphoinositide 3-kinases (PI3K) family of serine/threonine kinases. Moravek *et al* showed that inhibition of mTORC1 (one of two distinct mTOR complexes) blocks hCG-stimulated expression of *Star*, *Cyp11a1* and *3 β -Hsd*, as well as progesterone production¹³. This effect was also observed when human luteinized GCs were stimulated with forskolin, the pharmacologic activator of adenylate cyclase, implicating the mTOR signaling pathway as a novel mediator of LH/hCG and cAMP-mediated steroidogenesis. Another PKA target, the MAPK superfamily, includes the extracellular signal regulated kinases ((ERK1/2), Jun N-terminal kinases (JNKs), and p38. ERK1/2 and p38 pathway

inhibitors block phosphorylation of the cAMP-response element binding protein (CREB)¹⁴, a known target of cAMP/PKA and regulator of GC progesterone production¹⁵, implicating ERK and p38 pathways in CREB activation. Thus, the evidence points to PKA as a “master upstream kinase,” sufficient to stimulate intracellular GC signaling and steroidogenesis.

New insights regarding the role of activins

Activins are well-recognized for their importance in various intra-ovarian processes including augmentation of gonadotropin activity, follicle survival and growth (reviewed in ^{16,17}). More recent work has helped to elucidate the biological functions of the different activins on gonadotropin action. Chang *et al* reported that pretreatment of immortalized human GCs (SV40) with the three activin isoforms A, B, or AB suppressed basal- and gonadotropin-induced STAR and progesterone production^{18,19}, while no effects were observed with activin C. Activins exert their effects via binding to type I serine/threonine kinase receptors activin receptor-like kinases (ALKs); activin-induced downregulation of STAR appears to be regulated primarily via the ALK4-mediated SMAD signaling pathway. In the work by Chang *et al*, knockdown of SMAD4 (the common downstream SMAD) blocked activin-induced downregulation of STAR in human luteinized granulosa cells^{18,19}, and pretreatment with an ALK4 inhibitor or transfection with *ALK4*-specific siRNA, which abolished activin-dependent SMAD phosphorylation, blocked *STAR* mRNA expression¹⁸. This work indicates that activins A, B, and AB differentially activate canonical SMAD-dependent and other signaling pathways²⁰, which associate with other transcription factors to activate or repress genes important in the production of progesterone.

Notch, Wnt, and GATA signaling pathway interactions further regulate progesterone

The highly conserved Notch signaling pathway is an important regulator of follicular differentiation and proliferation. This family of proteins consists of four receptors (NOTCH1–4) and five ligands (Deltalike (*Dll*) 1, *Dll3*, *Dll4*, Jagged (*Jag*) 1, and *Jag2*). The NOTCH receptor-ligand interaction result in an intracellular signaling cascade involving proteolytic cleavage and release of the Notch intracellular domain (NICD), which acts as a transcription factor, translocating to the nucleus and activating the downstream repressors HES and HEY (for a review of Notch signaling pathway effectors, see ²¹). Recent studies have demonstrated the importance of Notch signaling in gonadotropin-dependent follicle development and luteal progesterone production. Accialini *et al* demonstrated that *in vitro* inhibition of Notch in cultured rat corpora lutea decreased P450_{scc} and progesterone production²², while Prasaya *et al* showed that Notch activity changes dynamically in the ovulatory time period, becoming significantly upregulated in mouse corpora lutea after hCG²³. When the same group, using a double reporter mouse model with fluorescence-labeled Notch-active and germ cells, ablated developing oocytes with the chemotherapeutic agent busulfan, Notch pathway activation in GCs was significantly reduced²⁴. In studies of Notch receptor and ligand expression in mouse ovary, *Jag1*, *Notch2* and *Notch3* increased post-hCG in mouse ovary, with localization of JAG1 shifting from oocytes to corpora lutea, post-hCG. In PMSG-primed cultured mouse GCs, knockdown of *Jag1* resulted in reduced *Star* and *Cyp11a1* as well as decreased progesterone production²³, and in cultured mouse GCs, recombinant JAG1 activates Notch target genes (and induces its own expression)²⁴. Oocyte-specific *Jag1* knockout mice also exhibit decreased Notch receptor expression²⁴.

These results are consistent with a novel link between the Notch signaling pathway, in particular the Notch ligand *Jag1*, and corpus luteum function, and further emphasize the importance of oocyte-secreted factors in GC function.

The highly conserved Wingless-type mouse mammary tumor virus integration site (Wnt) family signaling pathway has been well described in the ovary, and intact ovarian Wnt signaling is essential for ovarian follicular development^{25,26}. Activation of Wnt signaling pathways, which involves Wnt ligand binding to the seven-transmembrane receptor Frizzled, causes nuclear translocation of hypophosphorylated β -catenin, whose targets include the transcription factor steroidogenic factor 1 (SF-1). More recently, Wnt signaling has been implicated specifically in the maintenance of corpus luteum function, including luteinization and vascularization. Accialini *et al* demonstrated that *in vivo* ovarian inhibition of the Wnt signaling pathway via intrabursal injections of small molecule inhibitors resulted in impaired corpus luteum development, decreased STAR and circulating progesterone. Notably, this work also linked Wnt signaling to the critical process of luteal angiogenesis by demonstrating decreased expression of ovarian VEGF isoforms after Wnt pathway inhibition²⁷. Moreover, as further evidence of crosstalk between these important pathways, inhibition of Wnt/ β -catenin *in vitro* in cultured rat corpora lutea stimulated Notch signaling (via an increase in HES expression), suggesting a mechanism by which interactions between the critical Notch and Wnt pathways can promote progesterone signaling²⁸.

The GATA family includes six zinc finger transcription factors which are essential for multiple aspects of embryonic development. In particular, GATA4 and GATA6 are active during early ovarian development, with important roles in follicular assembly and GC differentiation, and GATA4 is a positive regulator of steroidogenic enzymes, activating *STAR*, *P450scc* and *3 β -HSD* promoters *in vitro* (reviewed in ²⁹). GATA family members can interact with Notch downstream effectors to potentiate steroidogenesis. In mice, targeted deletion of GATA4 and GATA6 in corpus luteum of mice results in decreased expression of *Star*, *3 β -Hsd*, *Cyp11a1*, and *Lhr* (the LH receptor), reduced serum progesterone, and infertility; deletion of either GATA4 or GATA6 individually results in subfertility³⁰. These effects of GATA4 on steroid enzyme promoters are nearly abolished after transfection with the activated Notch receptors NOTCH1ICD, NOTCH2ICD, and NOTCH3IC³¹ as well as by the Notch downstream effector HEY2³¹. Thus, the Notch signaling pathway appears to interact with GATA family members to further modulate steroidogenic gene expression and progesterone production.

Local and systemic growth factors modulate luteal progesterone production

In addition to somatic cell-derived factors, oocyte-secreted proteins are critical in the regulation of progesterone production by GCs. The transforming growth factor β (TGF β) superfamily is a large family of proteins which are expressed in the ovary and widely regulate multiple aspects of reproductive function. TGF β proteins are synthesized as prepropeptides, processed and secreted as homo- or hetero-dimers, at which point they activate transmembrane serine/threonine kinase receptors³². GDF-9, an oocyte-secreted paracrine factor in the TGF β family^{33–35}, is well known to act as an oocyte paracrine regulator of follicular progesterone production. GDF-8, another member of the TGF β

family, is found in GCs and follicular fluid, where GDF-8 concentrations inversely relate to progesterone levels³⁶. *In vitro* studies have demonstrated that GDF-8 suppresses progesterone, the LH receptor, and STAR in human luteinized GCs via multiple signaling intermediaries, including ALK5 and SMAD3 and ERK1/2 signaling pathways. It has been proposed that GDF-8's suppressive effects on GC progesterone production serve as a protective mechanism to prevent premature luteinization and progesterone elevation.

Neurotrophic factors such as brain derived neurotrophic factor (BDNF) are expressed in many organ systems including the nervous and reproductive systems. BDNF modulates GC sensitivity to FSH, thus indirectly regulating steroidogenesis³⁷. While BDNF alone did not stimulate progesterone in *in vitro* studies using human cultured granulosa KGN cells, BDNF + FSH activated the cAMP/PKA pathway and had a synergistic effect on progesterone production over FSH alone³⁷. Decreased FSH receptor (FSHR) expression and enhanced FSHR phosphorylation (indicating receptor desensitization) was also observed after BDNF + FSH treatment³⁷, suggesting that BDNF may act synergistically with FSH to promote ovarian progesterone production, but also serve as part of a negative feedback loop, preventing the further expression of FSHR and titrating the ovarian progesterone response. Lastly, the EGF-like family of growth factors (which includes EGF and the EGF-like growth factors amphiregulin (AREG), betacellulin (BTC) and epiregulin (EREG)) appears to have a luteolytic role in progesterone regulation. In primary luteinized human GCs, treatment with LH increases AREG^{38,39}, BTC³⁸, and EREG^{38,39} and promotes production of cyclooxygenase-2 (COX-2, which is essential for prostaglandin and luteal progesterone production). Inhibition of epidermal growth factor receptor (EGFR) abolishes these effects³⁹, suggesting that AREG, BTC, and EREG may act as intermediaries in LH-induced ovulation via regulation of COX-2. Follicular fluid AREG levels positively correlate with serum and follicular fluid progesterone³⁹.

Novel endocrine regulators of progesterone production: BMPs and orexins

Bone morphogenetic proteins (BMPs), also members of the TGF β superfamily, are not only critical for oocyte developmental competence, but also function in GCs to promote proliferation, differentiation, and steroid hormone production. In particular, BMPs appear to play physiological roles as luteinization inhibitors in growing follicles. BMPs exert their function in GCs via binding serine threonine kinase receptors; the activated BMP-receptor complexes initiate phosphorylation of downstream SMAD transcription factors, forming transcription-regulating complexes which localize to the nucleus and regulate the expression of target genes. These target genes include several related to GC luteinization and progesterone production⁴⁰. Like GDF-9, BMP-15 is a well-known oocyte-secreted factor which acts directly on GCs, signalling through its receptors as well as acting synergistically with GDF-9 to activate SMAD signaling⁴¹. More recently, BMP family members, including BMP-6 in GCs, have been shown to suppress STAR expression and inhibit FSH-stimulated progesterone production^{33,42}. This suppression is mediated via the BMP receptor ALK3 and involves enhanced SMAD1/5/8-SMAD4 signaling⁴³. Taken together, BMP signaling pathways appear to inhibit luteal progesterone synthesis by modulating ALK- and SMAD-dependent signaling pathways and suppressing GC FSH responsiveness.

Orexins, produced in the hypothalamus, have been linked broadly to hypothalamic-pituitary-ovarian (HPO) axis functioning at multiple levels in part via their effects on BMP receptor signaling. Orexins are neuropeptides which regulate multiple homeostatic functions including wakefulness, appetite, and energy balance. The actions of orexins are mediated by their receptors, OX1 and OX2, and include regulation of hypothalamic gonadotropin-releasing hormone (GnRH) induction and pituitary LH secretion as well as direct effects of orexins on ovarian steroid hormone production. In cultured rat GCs isolated from DES-treated rats, treatment with orexin A increased FSH-induced cAMP production, enhanced mRNA levels of steroidogenic enzymes including *Star*, *Cyp11a1* and *3 β -Hsd* and significantly increased FSH-stimulated progesterone synthesis in isolated rat GCs⁴⁴. Treatment with orexin A also suppressed BMP receptors and BMP signaling pathway factors including SMAD 1, 5, and 9; these actions were reversed in the presence of an orexin receptor antagonist⁴⁵. BMPs downregulate the expression of OX1 and OX2⁴⁵, suggesting that can BMPs modulate the sensitivity of GCs to orexin. Thus, orexins, by downregulating and suppressing the BMP signaling pathway, appear to modulate local effects of BMP and represent intriguing candidate regulators of GC luteinization.

Glucose and insulin signaling mediators

Insulin signaling, including pathways mediated by insulin-like growth factors IGF1 and IGF2, plays critical roles in ovarian steroidogenesis, providing an important mechanism for intrafollicular autocrine regulation of steroid hormone production (reviewed in ^{46–48}). Upon binding to their associated tyrosine kinases (insulin receptor (INSR) and growth receptor IGF1R)), downstream signaling cascades involving multiple signaling pathways including PI3K/AKT, MAPK, and ERK are activated⁴⁹. Recent studies using a *Pgr*-Cre driven GC-specific double knockout mouse model for INSR and IGF1R showed that both ovarian insulin receptors are essential for periovulatory follicular function. Double receptor knockout mice exhibited impaired oocyte development, ovulation, loss of key enzymes in the steroid synthesis pathway, reduced serum progesterone, and infertility. Singly, effects of *Igf1r* knockout were more prominent; these mice exhibited subfertility while *Insr* knockout mice had nearly normal litter sizes⁵⁰. In another recent study, disruption of insulin receptor substrate proteins, the classical substrates of the insulin and IGF1 receptor (specifically, insulin receptor substrate [IRS] 2) in cultured preluteinized mouse GCs decreased *Star* and progesterone in a PI3/AKT dependent manner⁵¹.

The incretins, a group of peptide hormones synthesized in the intestine, are another essential component of glucose homeostasis⁵². Incretins are secreted from the pancreas in response to a glucose challenge and stimulate insulin secretion to regulate glucose levels after a meal. *In vitro* studies suggest incretins may function directly to suppress ovarian progesterone production. Specifically, incretins appear to impair FSH-induced progesterone production by promoting GC BMP signaling. In isolated primary GCs from DES-treated rats, the incretins gastric inhibitory polypeptide (GIP) and glucagon like peptide (GLP-1) induce transcription of several mediators of progesterone production, including the BMP receptors *Alk3* and *Alk6*, the BMP target gene *Id-1*⁵², and promote phosphorylation of SMAD1/5/8, suppression of STAR, P450scc, 3 β -HSD, and progesterone production⁵². *In vivo* data is less clear. Luteinized GCs from women with PCOS produce decreased amounts of progesterone

in culture compared to non-PCOS women⁵³, but the mechanism for this is unclear. In some studies of obese women with PCOS, serum incretin levels were higher than in control women^{54–56}; however, other studies have found decreased levels⁸². Thus, while *in vitro* data suggests a modulatory effect of incretins on steroid hormone production, the precise role of these effects in the clinical setting has not been determined.

Emerging data also suggests roles for adipokines in the neuroendocrine control of steroid hormone production. The adipocyte-secreted cytokine leptin promotes satiety in part by altering the secretion of hypothalamic neuropeptides such as neuropeptide Y (NPY), a potent appetite stimulator (reviewed in ⁵⁷). Treatment of mice with either leptin or an antagonist to NPY increases serum LH but decreases serum progesterone⁵⁸. In contrast, in cultured follicular fluid GCs, leptin-specific siRNA knockdown increased progesterone production and reduced JAK2/STAT3 phosphorylation, suggesting an inhibitor effect of leptin on these signalling pathways⁵⁸. However, given the complex interactions between leptin and glucose/insulin signaling (e.g. homozygous leptin mutant mice exhibit hyperglycemia and hyperinsulinemia as well as infertility^{57,59}) and the known effects of altered insulin signaling on steroidogenesis, it is possible that leptin's effects on steroidogenesis can be attributed to indirect effects on insulin rather than direct modulation of steroid hormones. Apelin (APLN), another adipokine involved in the regulation of a broad range of physiological processes APLN is detectable in GCs and cumulus cells of large human follicles; treatment of primary luteinized human GCs with recombinant APLN increases 3 β -HSD and progesterone secretion both alone and in combination with IGF1. These positive effects require activation of the AKT and MAPK3/1 pathways⁶⁰. Women with PCOS have higher follicular fluid APLN concentration as compared to controls; these results can be further stratified by weight status, with obese PCOS women having higher APLN than non-obese PCOS women⁶⁰. However, mice lacking the *Apln* gene are fertile⁶¹; it has been suggested that this is due to redundancy between the *Apln* gene and other adipokines.

Vitamin D

An increasing body of literature suggests effects of vitamin D on progesterone production, though different model systems have yielded conflicting results. In human luteinized GCs, treatment with vitamin D increases 3 β -HSD mRNA and progesterone production⁶². However, in non-luteinized mouse GCs obtained from DHEA-induced PCOS mice, vitamin D downregulated *Cyp11a1*, 3 β -Hsd, and *Star* and decreased progesterone production^{63,64}. Steroidogenic enzyme expression in this model was upregulated at baseline compared to non-DHEA-treated mice⁶³. Nuclear localization of the BMP signaling factors SMAD1/5/8 was also significantly reduced by vitamin D⁶². These conflicting findings regarding the effects of vitamin D on progesterone may be attributable to potential species- and tissue-specific effects of vitamin D and highlights the difficulty in comparing results from luteinized, gonadotropin exposed cells as compared to a nonluteinized population of cells. It is clear that further investigation is required to determine whether and how *in vitro* effects of vitamin D translate to clinical use in the patient setting.

Beyond modulation of steroidogenic enzymes: the influence of intracellular stress signals

Control of reactive oxygen species (ROS) production and activity of antioxidant enzymes is essential for GC function, including progesterone production. Reactive oxygen species (ROS) are generated as byproducts during oxidative phosphorylation; when ROS are generated in excess of the cellular antioxidant capacity, subsequent oxidative stress results in cellular damage⁶⁵. In cultured human cumulus cells, inhibition of mitochondrial uncoupling protein 2 (which uncouples oxidative phosphorylation to regulate ATP synthesis) results in elevated levels of ROS, increased levels of the apoptosis marker caspase-3, and decreased progesterone, indicating that disruptions in oxidative phosphorylation dysregulate follicular steroidogenesis and survival^{66,67}. Silent information regulator genes (Sirtuins), another group of mitochondrial proteins, regulate ROS generation by deactivating targets involved in ROS production. Sirtuins are targets of the antioxidant polyphenol compound resveratrol, which inhibits proliferation and androgen production by theca-interstitial cells and decreases serum androgen in clinical studies of women with PCOS (⁶⁸ and reviewed in ⁶⁹), although older studies investigating resveratrol's effects on progesterone production *in vitro* are conflicting^{70,71}. The endoplasmic reticulum also responds to stress, triggering an accumulation of misfolded proteins in a protective response known as the endoplasmic reticulum (ER) stress response. Activation of this response initiates the unfolding protein response (UPR), a downstream signaling cascade, in an attempt to reestablish homeostasis⁷². Proper protein folding and an appropriate ER stress response is critical for normal corpus luteum function, and disruption of this process results in aberrant luteal steroidogenesis. For example, the sensor protein activating transcription factor 6 (ATF6) is a necessary component of ER homeostasis, transmitting stress signals during ER stress signaling^{73,74}. In mouse preleutenized GCs, knockdown of ATF6 (whose production is induced by both FSH and LH) causes decreased apoptosis, increased *Cyp11a1* and *Star*, and increased progesterone production, suggesting that loss of the ER-mediated (GC) stress response results in dysregulated steroidogenesis^{73,74}.

Structural disruption of luteal cytoskeletal function: new functions for RhoA

Ras homolog gene family member A (RhoA) is a small membrane bound GTP-ase which regulates the actin cytoskeleton and is highly expressed in murine luteal cells. El-Zowalaty *et al* recently described a novel function for RhoA in corpus luteum progesterone production. The group's studies using a conditional deletion of Rho in corpus luteum (*RhoA^{d/d}*) demonstrated that RhoA is critical for normal ovarian progesterone production; *RhoA* conditional knockout female mice ovulate normally but are infertile with defective corpus luteum function, including a dramatic downregulation of *STAR* mRNA and progesterone insufficiency⁷⁵. As a potential mechanism for decreased *Star*, mitochondrial density was found to be decreased in *RhoA^{d/d}* mice. The authors proposed that RhoA-mediated cytoskeletal function may facilitate cholesterol transport into mitochondria as well as luteal mitochondrial density itself; consequently, loss of *RhoA* would be expected to disrupt cytoskeletal function, lipid transport, and mitochondrial density. In turn, this leads to less available cholesterol substrate for progesterone production, and that negative feedback from decreased progesterone results in a compensatory decrease in *STAR* expression in *RhoA^{d/d}* mice⁷⁵. The signaling pathways involved in *RhoA*-mediated progesterone

regulation remain as yet unknown, and RhoA expression in human corpus luteum has not been described.

Emerging roles for noncoding RNAs in regulation of luteal progesterone

A significant portion of the genome encodes transcripts that are transcribed into functional RNA but not translated into protein. These noncoding RNAs (ncRNAs) have emerged as master regulators of growth and differentiation and are increasingly recognized for their roles in the regulation of a wide variety of biological functions, including steroid hormone production. Perhaps the best-characterized ncRNAs with respect to steroid hormone production are the microRNAs (miRNAs), 21–22 nucleotide ncRNAs that suppress gene expression by silencing mRNA translation or leading to target mRNA degradation. The expression of miRNAs fluctuates during follicular and luteal development⁸³; luteal functions which have been attributed to miRNAs include LH receptor downregulation (miR-122 and mir-136)^{76,77}, inhibition of apoptosis after the LH surge⁸⁶, and angiogenesis⁸⁷ in rodent models. More recently, miRNAs have been shown to have direct effects on expression of luteal steroidogenic enzymes. Hu *et al* showed that miR-132, which is expressed in cultured cAMP-treated rat GCs, decreases STAR protein levels and increased expression of 3 β -HSD and 20 α -HSD, leading to inactivation of progesterone via its conversion to the biologically inactive 20 α -OHP⁷⁸. In human granulosa lutein cells, knockdown of another miRNA, miR-96, inhibited progesterone production via the transcription factor FOXO1⁷⁹. These studies indicate that miRNAs may play key role in the direct posttranscriptional regulation of steroid hormone biosynthesis.

Long noncoding RNAs (lncRNAs) are another class of ncRNAs which can interact with DNA and RNA, acting as molecular sponges or regulators of transcriptional activity, although fewer studies exist on the role of lncRNAs with respect to steroidogenesis. Li *et al* recently showed that the steroid receptor RNA activator (SRA), which enhances transcription of steroid receptors including the progesterone receptor (reviewed in ⁸⁰), also increases expression of *Cyp11a1* and progesterone production in cultured primary mouse GCs *in vitro*⁸¹. The lncRNA *H19*, which has been recently studied as a regulator of follicular development and steroid-dependent processes including leiomyoma growth^{82–84}, represents another novel mechanism by which steroidogenic enzymes can be regulated at the post-transcriptional level. *H19* sequesters the miRNA let-7, a small molecule that predominantly functions to silence target genes^{85,86}; *Star* is one such let-7 target gene⁸⁷. Loss of *H19* *in vitro* in immortalized mouse granulosa (KGN) cells results in disrupted *STAR* production and progesterone synthesis⁸⁷. Furthermore, this novel regulatory mechanism is functional *in vivo*; loss of *H19* also disrupts ovarian *STAR* but also results in altered progesterone production in an *H19*KO mouse model⁸⁸. Nakagawa *et al* utilized a knockout mouse for another lncRNA, *Neat1*, which localizes to nuclear bodies and is expressed in corpus luteum, demonstrating that loss of *Neat1* results in infertility. While *Neat1* knockout mice had normal ovulation, the fertility defect was rescued by administration of progesterone⁸⁹, suggesting a critical role for *Neat1* in luteal function and progesterone production. Thus, it is becoming increasingly clear that ncRNAs, including lncRNAs, target various critical genes for supporting luteal progesterone production.

Potential Sources of Environmental Disruption to Progesterone Production

The number of the signaling pathways contributing to the production of progesterone is expansive. Thus, it is not surprising that this complex system is susceptible to disruption by exogenous chemicals that can mimic or interfere with the activities of endogenous hormones or their signaling pathways. Endocrine disrupting chemicals (EDCs) are defined by their ability to interfere with the normal functions of endocrine system. It is impossible to estimate the total number of chemicals in use today, although manufacturing trends suggests that the number has dramatically increased over the past 50 years. Unfortunately, testing for EDC activity has not kept up with these growing numbers, leading to the potential for exposure without a complete understanding of risk⁹⁰. The reviewed studies provide recent evidence demonstrating the variety of mechanisms by which EDCs can alter progesterone production (Figure 2 and Table 1).

Bisphenols

Bisphenol A (BPA) is one of the most well-studied EDCs, originally tested as an artificial estrogen in the 1930s⁹¹. Subsequently, manufacturing industries incorporated this monomer as a component of many consumer products, including plastic reusable bottles, medical devices, toys, sports equipment, thermal printer paper, water pipes, dental sealants, and food packaging. Reflecting the vast use of BPA in consumer products and its ability to be released from these materials, the National Health and Nutritional Examination Survey (NHANES) found in 2004 that >93% of the U.S. population had detectable levels of BPA in their urine⁹². The most common modality of exposure is by the consumption of BPA-contaminated foods, where BPA then passes into tissues and fluids throughout the body^{93,94}. In fact, BPA has been measured in follicular fluid at 10.5 nM⁹⁵, and BPA concentrations have been inversely correlated with ovarian response in women undergoing IVF cycles⁹⁶. However, *in vitro* studies analyzing the functional effect of BPA on progesterone synthesis have resulted in conflicting conclusions regarding the necessary concentration for effect and mechanism. In one study, exposure of human luteinized GCs isolated from women undergoing IVF to 8.7 μ M BPA significantly decreased progesterone production approximately 20%, while 87 μ M decreased progesterone production by approximately 75%⁹⁷. These concentrations of BPA were also associated with dose-dependent reductions in transcript levels of *3 β -HSD* and *CYP11A1*, although no effect was seen on *STAR* mRNA expression. Studies using the structural analog of BPA, bisphenol S (BPS), reported a similar decrease in progesterone production and *CYP11A1* expression when luteinized GCs isolated from women undergoing IVF treatment were exposed to 10 or 50 μ M BPS⁹⁸. Conversely, BPA at higher concentrations had the opposite effect on progesterone production in cultured human cumulus GCs, which reflect the properties of GCs in the early antral follicle⁹⁹. This study determined that BPA increased progesterone production via an EGFR- and ERK1/2-mediated rise in *STAR* transcript levels and suggests that the effect of BPA on progesterone production can depend on BPA concentration and the differentiation status of GCs. Interestingly, studies using isolated, immature rodent GCs demonstrated that progesterone production was attenuated following 48 hr exposure to 100 μ M BPA, although this reduction was associated with increased mRNA expression of *Cyp11a1*, *3 β -Hsd*, and *Star*, similar to what was described for human cumulus GCs but

contrary to that in human luteinized GCs¹⁰⁰. The effect of BPA on progesterone synthesis in isolated, immature rodent GCs was found to be mediated through alterations to cholesterol homeostasis, confirmed when progesterone production was partially restored by the addition of exogenous cholesterol¹⁰⁰. The discrepancies between these studies may be rooted in various differences in study design. Mansur *et al.* noted that their results may have been influenced by the use of cells that were previously exposed to gonadotropic stimulation¹⁰¹. Furthermore, the range of BPA concentrations tested differed between studies. For example, the lowest concentration where BPA induced *Star* mRNA expression in rat GCs was 100 μ M, a concentration not tested with human luteinized GCs. Most importantly however, differences between studies may reflect species-specific sensitivity to BPA.

In vivo studies have reported equally conflicting results. Early postnatal exposure to BPA or BPS was associated with a dose-dependent decrease in progesterone levels in adult rats¹⁰². Rats chronically treated as adults for 9 days with BPA doses of 25 mg/kg also demonstrated markedly decreased levels of progesterone synthesis, where it was suggested that the generation of reactive oxygen species and associated oxidative stress was the primary cause of BPA-mediated effects on ovarian function and progesterone production¹⁰³. As evidence to this hypothesis, BPA was found to decrease the activity of superoxide dismutase and catalase, important mediators in the defense against oxidative stress, and increase levels of nitrous oxide and malondialdehyde, markers of oxidative stress^{104,105}. Similar results were found when neonatal ovaries from mice were cultured for 8 days with BPA^{106,107}. Interestingly, the antioxidant properties of ginseng extract (*Panax ginseng*) were able to completely rescue the progesterone-lowering effects of BPA in pregnant rats, suggesting a potential mechanism to combat the toxic effects of BPA¹⁰⁸. In contrast to these *in vivo* studies, results from the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY_BPA) program indicated that early-life exposure to BPA had no long-term effects on the production of progesterone in rats¹⁰⁹. However, data from CLARITY-BPA did show a non-significant decrease in serum progesterone levels with continuous exposure to BPA, comparable to that reported by Banerjee *et al.* It is intriguing to consider that many of the endpoints evaluated in the CLARITY-BPA program displayed a nonmonotonic relationship with dose, including a variety of low-dose effects. Risk assessment has traditionally relied on a dose-response relationship, and discrepant results across BPA exposure studies demonstrate that EDCs do not follow typical toxicology rules and that the study design can strongly influence results¹¹⁰.

Phthalates

Phthalates, commonly used in manufacturing as plasticizers, solvents, and as excipients for drug formulation, are considered the “everywhere chemical” due to their presence in a number of products encountered on a daily basis. As plasticizers, phthalates are used in food packaging, children’s toys, flooring, and medical equipment, and as solvents, phthalates are found in soaps, shampoos, lotions, perfumes, hair sprays, and nail polishes^{111,112}. Some phthalates are included as a constituent of the “inactive ingredients” in drug formulations for the purpose of drug delivery via controlled release or to maintain the stability of a dose. However, new guidelines from the FDA now recommend the discontinuation of certain phthalates in prescription and non-prescription products. Phthalates are non-covalently

bound to plastics, indicating that they leach from these products and can be easily absorbed through ingestion, inhalation, and dermal exposure^{113,114}. Interestingly, women are reported to have higher urine levels of phthalates than men, with detectable levels in the placenta, amniotic fluid, cord blood, breast milk, and follicular fluid (approximated to be 15 ng/mL in human follicular fluid)^{112,115,116}. Studies in both isolated human cells and animal models have demonstrated that phthalates can alter progesterone production and the expression of associated steroidogenic enzymes, with the effect dependent on timing and exposure¹¹⁷.

Di-2-ethylhexyl phthalate (DEHP) is estimated to have the highest environmental prevalence of all phthalate esters, with exposure rates of 30 µg/kg/day in the general population and up to 700 µg/kg/day for occupationally exposed workers^{113,118}. In rats, treatment of GCs isolated from preovulatory cumulus-oocyte complexes with 400 µM DEHP decreased progesterone production and reduced *Cyp11a1* and *Star* mRNA expression¹¹⁹. However, it is unclear whether the decrease in progesterone production was directly attributed to DEHP or the impact of DEHP on GC viability, as the authors also reported increased levels of oxidative stress, hypoxia, reactive oxygen species, and cellular senescence, which was partially attenuated by co-treatment with N-acetyl-cysteine (NAC), an antioxidant that scavenges free radicals¹²⁰. An earlier study found that lower concentrations of DEHP (1 nM) were sufficient to reduce basal and hCG-stimulated progesterone release without altering the cellular survival of GCs isolated from IVF patients and luteal cells cultured from women characterized with normal cycles^{121,122}. Thus, DEHP may directly impair progesterone production.

In vivo studies are less clear. For example, adult mice treated for 10 days with 500 mg/kg DEHP and evaluated 9 months later showed higher levels of progesterone, fewer follicles, and altered estrous cyclicity, leading the authors to hypothesize that DEHP caused accelerated reproductive aging¹²³. However, a 16-week, long-term exposure of mice to the same concentration of DEHP also disrupted estrous cyclicity and induced apoptosis in the ovary but decreased serum progesterone levels, indicating that the exposure period or length may dictate the overall impact on progesterone production¹²⁴. Modeling the effects of phthalates based on the timing of exposure is essential, especially for vulnerable populations. Medical therapies and equipment (e.g. tubing and IV bags) represent one of the highest means of exposure, and DEHP metabolite levels were reported to be 14 times higher in infants in intensive neonatal care units compared to infants in low-intensive units¹²⁵. When DEHP exposure occurs early through the lactating dam, female offspring demonstrate significantly lower serum progesterone levels and decreased ovarian expression of *Star* and *3β-Hsd* as adults¹²⁶. For mice exposed to DEHP *in utero*, serum levels of leptin are decreased in offspring, suggesting that emerging mediators of progesterone production may also be sensitive to DEHP¹²⁷. In agreement with these animal model studies, a prospective cohort study found an inverse correlation between maternal DEHP levels and leptin levels in cord blood from female neonates¹²⁸. Other emerging regulators of progesterone production are also sensitive to DEHP, as a 6-week exposure to DEHP in mice was found to increase negative regulators of follicular GC proliferation: let-7b, miR-17-5p, miR-181a, and miR-151 in the ovary¹²⁹. Finally, in studies where the *in utero* DEHP exposure is followed via transgenerational analysis, the F1 generation is characterized by accelerated reproductive

aging, as indicated by decreased total follicle counts, and decreased progesterone levels are found in the F2 generation, demonstrating the lasting impact of DEHP exposure¹³⁰.

Di-butyl phthalate (DBP) is another commonly used phthalate, with an estimated daily human exposure rate of 0.84–5.22 µg/kg/day¹³¹. DBP has been shown to cause a dose-dependent reduction in progesterone production using *ex vivo* cultured ovaries from chronically exposed adult rats¹³². Primary cultures of mural GCs isolated from women undergoing IVF treatment also found that 359 nM DBP reduced progesterone production, in addition to decreased expression of *STAR* and *CYP11A1*, while a previous study established that 10 nM was sufficient to reduce progesterone production in luteinized GCs isolated from women with reported normal menstrual cycles^{122,133}. It is important to note that phthalates can be readily metabolized, and the metabolites have also been measured in follicular fluid¹³⁴. Thus, the cumulative physiological effect of phthalates will likely reflect the EDC activities of the parent compound and all toxic metabolites.

Perfluoroalkyl Substances (PFAS)

Much like the bisphenols and phthalates, exposure to PFAS is nearly ubiquitous, and an estimated 98% of the U.S. population has detectable levels of PFAS in their serum^{135,136}. Chronic exposure to perfluorooctane sulfonate (PFOS) at a concentration of 0.1 mg/kg in mice caused decreased levels of serum progesterone, fewer mature follicles and corpora luteum, and decreased ovarian *Star* transcript levels¹³⁷. The concentration of GnRH in the hypothalamus, activation of hypothalamic neurons, and levels of gonadotropins were also significantly reduced in PFOS-exposed mice, which demonstrates that PFOS can impact progesterone production through a variety of mechanisms⁹. In women, serum PFOS concentrations have been found to be inversely correlated with salivary progesterone levels¹³⁸. Accordingly, a prospective birth cohort also reported an inverse correlation between serum PFOS and progesterone levels in mothers and female infants¹³⁹. Studies determining the mechanisms by which perfluoroalkyl substances alter progesterone levels in humans are lacking; thus, it is not clear whether the effect of PFOS on progesterone in these cohort studies is direct at the level of the ovary or through modulation of the HPO axis.

Pesticides

The chemical class of “pesticides” covers insecticides, fungicides, herbicides, and rodenticides, which have been essential to controlling agricultural pests and securing the food supply as the worldwide population expands. Originally developed in the late 1800’s, dichlorodiphenyltrichloroethane (DDT) was found to be an extremely useful insecticide, preventing the spread of insect-borne diseases, such as typhus and malaria¹⁴⁰. However, in 1962, Rachel Carson’s “Silent Spring” documented the adverse effects of DDT, leading to a nationwide ban and sparking the environmental movement¹⁴¹. Since that time, a variety of pesticides have been shown to impair female reproduction through direct effects on the ovary and progesterone production.

The organochlorines (e.g. DDT and methoxychlor) constitute one of the major classes of pesticides and are known for their high toxicity, bioaccumulation, and environmental persistence¹⁴². Serum DDT levels have been associated with decreased progesterone levels

and a shorter luteal phase in women, which was supported by studies in human cumulus cells that demonstrated diminished hCG-supported progesterone production with DDT exposure^{143,144}. Likewise, methoxychlor significantly decreased serum progesterone levels in rats after oral dosing twice a week for 10 months¹⁴⁵. A suppressive effect on progesterone production was also evident with the methoxychlor metabolite HPTE¹⁴⁶. While not exerting long-term effects on serum progesterone levels, *in utero* and early post-natal exposure to methoxychlor did cause premature termination of estrous cycles, indicative of reproductive senescence, in adult rats¹⁴⁷. Organophosphates are another class of pesticide, thought to be less persistent in the environment than organochlorines but with equal or greater toxicity¹⁴⁸. A single dose of the organophosphate diazinon was found to decrease progesterone production and ovarian *Star* expression, leading to much smaller corpus lutea in rats¹⁴⁹. These studies suggest that organochlorines and organophosphates exert similar negative effects on the signaling pathways leading to progesterone synthesis in the ovary.

Pyrethroids represent a new class of pesticides, being preferred over organochlorines and organophosphates due to their efficacy and relatively low toxicity¹⁵⁰. Despite the improved toxicity profile, pyrethroids also demonstrate EDC activity¹⁵¹. The synthetic pyrethroid lambda cyhalothrin was found to reduce serum progesterone levels, decrease ovarian *Star* and *17b-hsd* expression, and diminish the activity of 17 β -HSD and 3 β -HSD in the rat ovary¹⁵². Fenvalerate, a synthetic pyrethroid insecticide, was also found to reduce basal progesterone production and *Cyp11a1* expression in cultured GCs isolated from immature rat follicles¹⁵³. Interestingly, paternal fenvalerate exposure augmented estrous cycle length and significantly increased serum progesterone concentrations in female offspring (F1), suggesting that exposure can have both direct and indirect effects passed down to subsequent generations¹⁵⁴.

Metals

Heavy metal contamination is an expanding problem for large parts of the developing world, with serious adverse effects on the reproductive health of both humans and animals¹⁵⁵. Epidemiological studies have demonstrated a correlation between heavy metal levels and spontaneous abortion, as well as an inverse correlation between heavy metals (specifically cadmium, zinc, copper, and lead) and serum progesterone levels¹⁵⁶. Metals are a unique environmental contaminant, as they are naturally occurring elements with physiological functions in plants, animals, and humans¹⁵⁷. For example, zinc is necessary for the functions of enzymes and transcription factors important to ovarian biology, but levels of zinc outside the homeostatic range can alter progesterone production and fertility^{158–162}. Depletion of zinc in mouse cumulus-oocyte complexes, using a transition metal chelator with a high affinity for zinc, enhanced progesterone production and was associated with elevated transcript levels of *Star* and *Cyp11a1*¹⁶³. A similar effect on *Star* expression was found in cumulus-oocyte complexes when mice were fed a zinc-deficient diet for 10 days. In this study, the authors hypothesized that a zinc-binding transcription factor repressed the expression of steroidogenic enzymes. Zinc deficiency may also impair the expression of novel progesterone production regulators *Gdf9*, *Igf2*, and *H19*, as mice placed on a zinc deficient diet prior to mating showed decreased expression of these genes in oocytes and developing blastocysts¹⁶⁴. Conversely, zinc administration in rats increases the hypothalamic

expression of novel progesterone regulator orexin, although these results have only been demonstrated for males^{165,166}. Further studies are required to identify how zinc regulates progesterone production, as most studies evaluating the mechanistic role of zinc in steroidogenesis have focused on effects in the male reproductive tract¹⁶⁷.

The effects of cadmium exposure on progesterone production have been extensively studied *in vivo* and *in vitro*, although the results suggest that the observed effects are dependent on dose, route of administration, timing of exposure, and species^{168–172}. For example, rat ovaries cultured *ex vivo* following a 6-week exposure to 0.25 mg/kg cadmium produced less progesterone¹⁷¹. Similarly, pigs supplemented with dietary cadmium showed an inverse correlation between serum progesterone levels and cadmium concentration¹⁷⁰. Yet, rats exposed to 0.09, 1.8, or 4.5 mg/kg of cadmium for ten days saw a significant increase in serum progesterone levels after 90 days, irrespective of the dose¹⁶⁸. Transgenerational studies have shown that when pregnant rats are exposed to cadmium during gestation and lactation, the F1 generation has dramatically increased serum progesterone levels and elevated expression of ovarian STAR, CYP11A1, and 3 β -HSD enzymes¹⁷³. The F2 generation also had higher serum progesterone levels, accompanied by augmented expression of ovarian CYP11A1.

An important conclusion from investigating the effects of EDCs on progesterone production is that the dose, timing, and length of exposure is a critical determinant of outcome. Thus, future experimental design should include a concentration range that covers the estimated environmental exposure and models exposures across the lifespan. It will also be important to consider environmentally relevant mixtures. While initial studies require chemicals to be studied individually in order to ascribe an evidence-based conclusion regarding their EDC activity, there is a need to go beyond this current scientific paradigm to incorporate the evaluation of chemical mixtures, which more closely reflect physiological exposures. It is possible that the combination of low-dose chemicals acting on the same pathway is greater than either chemical alone⁹⁰. For example, employing a combination of phthalates and alkylphenols, in concentrations less than those found to produce effects when studied individually, caused a significant decrease in both progesterone production and the expression of steroidogenic enzymes¹⁷⁴. The progression of studying individual chemicals to chemical mixtures will further our mechanistic understanding of how real-world exposures cause harm to female fertility.

Conclusions

Progesterone is a critical sex steroid hormone which plays important roles in luteinization and maintenance of pregnancy and serves as a precursor for other essential steroids such as glucocorticoids and androgens. Several reproductive disorders, including miscarriage, implantation failure and polycystic ovary syndrome are characterized by perturbations in progesterone levels. Both classical and novel endocrine mechanisms, as well as local factors in the ovary, via paracrine and autocrine mechanisms, are important for production of progesterone. The use of *in vitro* and *in vivo* models, human cell lines and luteinized cells obtained from artificial ovarian stimulation cycles, and non-human luteinized and non-luteinized cell lines and tissues contributes to the heterogeneity of data available. What is

clear is that the body of literature regarding the complex regulation of progesterone is expanding rapidly, and the precise regulatory factors identified require further study.

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Highlights:

- While gonadotropin-mediated pathways are recognized as broad mediators of steroidogenic signaling pathways, the mechanisms driving granulosa cell progesterone production are much more intricate than previously recognized, with both local and systemic factors interacting to promote successful GC function and physiological fine-tuning of progesterone levels.
- Additionally, a growing body of evidence suggests that exogenous compounds known as endocrine disruptors can cause adverse effects on progesterone production, reproduction, and fertility, a topic which has garnered increasing attention, and controversy
- Here, we will highlight new observations regarding mediators of luteal progesterone production, focusing on integrating findings into a comprehensive network, when possible. We will also review recent evidence regarding the effect of environmental and endocrine disrupting compounds on progesterone production and discuss areas of potential future investigation which may help clarify these effects

Figure 1. Interactions between novel mediators of luteal progesterone production in the pre- and post-ovulatory follicle.

In the pre-ovulatory follicle, granulosa-cell-produced signaling pathways including cAMP/PKA, AMP, ERK1/2, CREB, and insulin signaling pathways are active. Preovulatory oocytes produce GDF9, BMP15, and JAG among others, which further interact with GCs. Regulatory roles have also been proposed for GATA4 and 6, ATF6, and vitamin D (suppressive). After ovulation, a complex interplay of factors serve to further regulate NOTCH signaling. BDNF, orexins, and incretins modulate FSH signaling, while *RhoA* modulates cholesterol transport. Factors including BMP6, GDF8, and lncRNAs/miRNAs further regulate steroidogenesis directly at the level of *Star* and *3b-HSD*. Lastly, factors such as the LH-mediated AREG, Wnt, and Vitamin D contribute to angiogenesis and CL maintenance.

Red line: decreases P4

Green line: increases P4

Purple line: no effect on P4

|||||: Transgenerational effect

→: upregulates

—|: downregulates

Thick line: finding based on 2 or more studies

Multiple branches: different findings from different studies

Figure 2.

Pictorial representation of EDC effects on various mechanisms of progesterone production.

Table 1.

Summary of recent studies investigating associations between endocrine disrupting chemicals and progesterone production.

Chemical (dose of effect)	Model (duration of exposure)	Effect	Reference
BPA (8.7 μ M) (or 2.0 and 20 mg/ml)	human luteinized granulosa cells-IVF (48 hr)	<ul style="list-style-type: none"> decreased progesterone Reduced <i>3β-HSD</i> and <i>CYP11A1</i> 	97
BPA (100 μ M)	human cumulus granulosa cells (48 hr)	<ul style="list-style-type: none"> increased progesterone production EGFR- and ERK1/2-mediated rise in <i>Star</i> 	99
BPA (100 μ M)	Immature rat granulosa cells (48 hr)	<ul style="list-style-type: none"> decreased progesterone increased <i>Cyp11a1</i>, <i>3β-Hsd</i>, and <i>Star</i> altered cholesterol homeostasis 	100
BPA (25 mg/kg/d)	Rats in vivo (9 days)	<ul style="list-style-type: none"> decreased progesterone increase in oxidative stress markers 	103
BPA (2.5, 25, 250, 2500, and 25,000 mg/kg/d)	Rats in vivo (gestation day 6 until 1 year)	<ul style="list-style-type: none"> no significant effect on progesterone 	109
BPA (0.5mg/kg/d)	Neonatal rats in vivo (PND1 to PND10)	<ul style="list-style-type: none"> decreased progesterone lower number of corpus lutea 	102
BPS (5mg/kg/d)	Neonatal rats in vivo (PND1 to PND10)	<ul style="list-style-type: none"> decreased progesterone lower number of corpus 	102
BPS (10 or 50 μ M)	luteinized GCs isolated from women undergoing IVF (48 hr)	<ul style="list-style-type: none"> decreased progesterone reduced <i>Cyp11a1</i> 	98
Ginseng extract (<i>Panax ginseng</i>)	Pregnant rats (pregnancy day 0 to 20)	restored progesterone levels in BPA or DEHP treated rats	108
DEHP (400 μ M)	rat granulosa cells (72 hr)	<ul style="list-style-type: none"> decreased progesterone reduced <i>Cyp11a1</i> and <i>Star</i> increased levels of oxidative stress 	119,175
DEHP (1 nM)	hCG-stimulated granulosa cells isolated from IVF patients (72 hrs)	decreased progesterone	121
DEHP (1 nM)	hCG-stimulated luteal cells from normally cycling women (24 hr)	decreased progesterone	122
DEHP (500 mg/kg/d)	In vivo mice (10 days- evaluated after 9n months)	<ul style="list-style-type: none"> increased progesterone fewer follicles altered estrous cyclicity 	123
DEHP (200mg/kg/d)	In vivo pregnant CD-1 mice (gestational day 11 until birth)	<ul style="list-style-type: none"> accelerated reproductive aging in the F1 generation decreased progesterone in F2 generation 	130
DEHP (10 mg/kg/d)	In vivo lactating mice (postnatal day 1 to 21)	<ul style="list-style-type: none"> In F1 generation: decreased progesterone decreased <i>Star</i> and <i>3β-Hsd</i> 	126
DBP (359 nM)	mural granulosa cells isolated from women undergoing IVF (48 hr)	<ul style="list-style-type: none"> decreased progesterone production decreased <i>STAR</i> and <i>CYP11A1</i> 	133
DBP (10 nM)	hCG-stimulated luteal cells from normally cycling women (24 hr)	decreased progesterone	122
Methoxychlor (20 μ g/kg or 100 mg/kg)	In vivo rats (embryonic d 19 through postnatal d 7)	<ul style="list-style-type: none"> no long-term effect on progesterone accelerated reproductive aging 	147
HPTE (100 nM)	In vitro rat luteal cells (24 hr)	<ul style="list-style-type: none"> decreased progesterone decreased P450_{scc} enzymatic activity 	146
Methoxychlor (200 mg/kg)	In vivo rats (twice a week for 10 months)	decrease progesterone	145
PFOS (0.1 mg/kg/d)	In vivo mice (4 months)	<ul style="list-style-type: none"> decreased progesterone lower number of corpus lutea decreased <i>Star</i> decreased FSH and LH 	137

Chemical (dose of effect)	Model (duration of exposure)	Effect	Reference
Diazinon (70 mg/kg)	In vivo immature rats (single dose)	<ul style="list-style-type: none"> • decreased progesterone • decreased <i>Star</i> • smaller corpus lutea 	149
Lambda cyhalothrin (6.3 mg/kg/d)	In vivo rats (14 days)	<ul style="list-style-type: none"> • decreased progesterone • decreased <i>Star</i> and <i>17β-Hsd</i> • diminished the activity of 17 β-HSD and 3β-HSD 	152
Fenvalerate (25μM)	In vitro rat granulosa cells (24 hr)	<ul style="list-style-type: none"> • decreased progesterone • decreased <i>Cyp11a1</i> 	153
Fenvalerate (10 mg/kg/d)	Paternal exposure (30 days)	<ul style="list-style-type: none"> • female offspring: • increased progesterone • increased the length of estrous cycle 	154
Cadmium (0.09 mg/kg/d)	Ex vivo rats (10 days – measured after 90 days)	<ul style="list-style-type: none"> • increased progesterone 	168
Cadmium (1mg/kg/d)	In vivo pregnant rats (gestation and lactation)	<ul style="list-style-type: none"> • F1 generation • increased progesterone • increased STAR, CYP11A1, and 3β -HSD enzymes • F2 generation: • increased progesterone • increased CYP11A1 	173
Cadmium (0.25 mg/kg/d)	Ex vivo rats (5 days a week for 6 weeks)	<ul style="list-style-type: none"> • decreased progesterone 	171
Cadmium (10mg/kg/d)	In vivo pigs (83 days)	<ul style="list-style-type: none"> • decreased progesterone • decreased FSH and LH 	170
Combination of DEHP, DBP, BBP, NP, and OP (1mg/kg/d)	In vivo pregnant mice (from 0.5 postcoital day during pregnancy and lactation)	<ul style="list-style-type: none"> • decreased progesterone • decreased <i>STAR</i> 	174