Endocannabinoid System in Pregnancy Maintenance and Labor: A Mini-Review

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The endocannabinoid system is a cell-signaling system present in multiple organ systems and is an integral part of sustaining the microenvironment necessary for early pregnancy success and maintenance. It plays a significant role in embryo development, transport and implantation as well as placentation. The current theory behind the initiation of term labor is that it is a complex, multifactorial process involving sex steroid hormones, prostaglandin production and interplay at the maternal-fetal interface resulting in increased expression of receptors and gap junctions that promote uterine activation. There is increasing evidence that, in addition to early pregnancy events, the ECS plays a regulatory role in pregnancy maintenance and the timing of labor. This review presents an overview of the ECS in pregnancy that focuses on late gestation and parturition.

Keywords: endocannabinoid system, myometrium, labor, parturition, anandamide, cannabinoid receptor, preterm labor, pregnancy

INTRODUCTION

The endocannabinoid cell-signaling system is based upon eicosanoid derivatives that promote cellular return to homeostasis in multiple organ systems and modulates smooth muscle function, metabolism of tissues, and immune function (1). The ECS includes CB₁ (CB₁R) and CB₂ (CB₂R) cannabinoid receptors, the endocannabinoid agonists anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and the enzymes that synthesize and metabolize the endocannabinoid ligands (1). CB₁R is a G protein-coupled receptor encoded by the CNR1 gene, the activation of which couples predominantly to Gₛ/G₁₀ proteins to promote effects on calcium channels, mitogen-activated protein kinases (MAPKs) and adenylyl cyclase (2, 3). Cannabinoid

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, anandamide; CB₁R, CB₁ cannabinoid receptor; CB₂R, CB₂ cannabinoid receptor; COX-2, cyclooxygenase-2; CRIP1ta, cannabinioid receptor interacting protein 1α; ECS, endocannabinoid cell-signaling system; FAAH, fatty acid amide hydrolase; MAPK, mitogen-activated protein kinase; OTR, oxytocin receptor; PG, prostaglandin; PEA, palmitylethanolamide; OEA, oleoylethanolamide; NAPE-PLD, N-acylphosphatidylethanolamine-specific phospholipase D; meth-AEA, methanandamide; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; Δ⁹ THC, Δ⁹ tetrahydrocannabinol; TRPV1, transient receptor potential vanilloid receptor type 1; MAGL, monoacylglycerol lipase; DAGL, diacylglycerol lipase; LPS, lipopolysaccharide; 5,6-EET-EA, 5,6-epoxyeicosatrienoic acid ethanolamide; PR-A, progesterone receptor A; PR-B, progesterone receptor B; IHC, immunohistochemistry.
receptor interacting protein 1a (CRIP1a) is a CB1R-associated protein that modulates trafficking of newly synthesized CB1Rs to the cell surface and attenuates receptor internalization (4).

Alterations in ECS signaling have been associated with early pregnancy loss (5). As recently reviewed, evidence supports significant contributions of the ECS in early pregnancy events including embryo transport, embryo implantation and placentation (6–8). There is also evidence supporting interplay among the sex steroid hormones, estrogen and progesterone, and the ECS (9, 10). Furthermore, inflammatory conditions in reproduction, including preeclampsia, miscarriage and endometriosis, have been associated with aberrant ECS signaling (11).

Pregnancy is considered a progesterone-dominant state, as progesterone is the major steroid hormone that contributes to the maintenance of pregnancy (12). Prior to the onset of labor, the uterus converts from a quiescent state to an active contractile state. The quiescent phase is maintained by progesterone and other factors that regulate contractile gene expression. In late pregnancy, there is an increase in estrogen and, with a growing fetus, an increase in myometrial stretch. This leads to increased expression of genes and receptors required for uterine contractions including prostaglandins (PG), connexin 43, and oxytocin receptor (OTR). Labor is a regulated inflammatory event during which prostaglandins PGF$_{2\alpha}$ and PGE$_2$ contribute to cervical ripening and enhance uterine contractions. Timing of normal labor requires communication between the fetal and maternal units. A similar pathway to labor is apparent in patients with preterm labor, although, the etiology and phenotype of preterm labor differs (13). This review will focus on the influence of the ECS on pregnancy maintenance and the timing of labor.

ENDOCANNABINOID INFLUENCE IN PREGNANCY AND LABOR

Several research groups have evaluated plasma AEA levels in pregnancy and in labor, reporting that plasma levels of AEA are predictive of the onset of parturition (14, 15). AEA is synthesized by N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD), as are other fatty acid ethanolamides such as oleoyl ethanolamide (OEA) and palmitoyl ethanolamide (PEA). Each of these are degraded primarily by fatty acid amide hydrolase (FAAH). Of these, only AEA is an agonist for the cannabinoid receptors, and AEA can be oxidized by cyclooxygenase-2 (COX-2) to PG-ethanolamides (prostamides) (16). AEA levels have been shown to be higher in the estrogen-dominant phases than in the more progesterone-dominant phases of the menstrual cycle (14). Luteal phase AEA levels are similar to those in the first trimester of a successful pregnancy (14). In the progesterone-dominant state of pregnancy, plasma AEA levels decrease in the second and third trimesters (Figure 1) (14). Plasma AEA levels increase just prior to the onset of labor, followed by a significant increase in labor (14). The source of this AEA is postulated to be a response of the endothelial cells to estradiol (17). Figure 1 shows AEA fluctuations as described along with the relative changes in progesterone and estrogen throughout pregnancy.

In addition to spontaneous labor, plasma AEA levels have been evaluated in induced labor (15). Nallendran and colleagues evaluated the percentage change in plasma AEA from a non-laboring to laboring state and determined AEA influence on induction-to-delivery interval (15). Their longitudinal observational study of 64 women showed a 1.5-fold increase in plasma AEA levels in labor. Furthermore, higher percentage rises in AEA were associated with shorter induction-to-delivery intervals.

Attempts to identify a marker that is reliably predictive of preterm delivery have been made, but current testing methods remain limited. The current standard of care for predicting preterm birth includes sonographic measurement of cervical length along with assessment of cervicovaginal fetal fibronectin (18). However, the positive predictive value of these tests is not ideal, as at least half of all women admitted for preterm labor end up delivering at term (18–20). The obstetric community remains in search of a non-invasive, reliable test to predict preterm birth. Bachkangi and colleagues investigated plasma levels of AEA, OEA, and PEA as potential markers of spontaneous preterm birth (21). They found plasma levels of AEA and PEA better predicted preterm birth in a high-risk population of women than the current standard methods. Additionally, plasma AEA levels predicted the gestational age of delivery.

AEA has been correlated with the expression of OTR in human placentas (22). Cells isolated from human placentas collected at term were cultured with methandandamide (methyl-AEA). OTR mRNA was increased in the placental cells exposed to methyl-AEA, and oxytocin concentrations were higher in the culture medium. These researchers proposed that AEA contributes to OTR expression and oxytocin release in labor (22). Yulia and colleagues investigated human primary myometrial cells isolated from uterine samples obtained at the time of cesarean section to determine the effect of cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) function on OTR expression (23). They identified an overall reduction in the cAMP/PKA pathway in late gestation and in labor to be associated with an increase in OTR mRNA and protein.

In ULTR myometrial cells, AEA-stimulated CB1R coupled to G$_{\alpha_{i/o}}$, inhibiting adenylyl cyclase and decreasing intracellular cAMP (24). CB1R also mediated a time- and concentration-dependent increase in ERK phosphorylation (24). Exposure to high levels of AEA decreased cell numbers and changed morphology typical of apoptotic cell death, which could be prevented by a CB1R antagonist or MAPK inhibitor (24). This AEA- and CB1R-mediated, ERK-dependent reduction in cell viability suggests a potential physiologic relevance of AEA in decidual senescence and in postpartum uterine involution. AEA is known to act not only on CB1R, but also other receptors including the ligand-gated transient receptor potential vanilloid receptor type 1 (TRPV1) (25) and peroxisome proliferator-activated receptors (PPARs) (26, 27). While these receptors have been identified in reproductive tissues, the role of AEA-mediated activity has not yet been clearly elucidated (24, 28, 29).
AEA and 2-AG stimulate PGE$_2$ production by fetal gestational membranes (30). In human placentas obtained at term, CP55940 (an agonist) stimulation of CB$_1$R resulted in a significant increase in PGE$_2$ by the amnion and chorion but a decrease in PGE$_2$ in the decidua (30). This increase in PGE$_2$ occurred through induction of COX-2 expression in amnion and chorion (30).

### CANNABINOID RECEPTOR INFLUENCE IN PREGNANCY AND LABOR

CB$_1$R and CB$_2$R are expressed in human uterine and placental tissue as outlined in Table 1. Dennedy and colleagues studied uterine contractility in segments of human myometrial tissue from the upper midline portion of the lower uterine segment obtained from elective cesarean delivery at term (31). They identified the presence of CB$_1$R and CB$_2$R mRNA in uterine tissue and observed a relaxant effect of AEA and $\Delta^9$ tetrahydrocannabinol ($\Delta^9$ THC) on myometrium. Using CB$_1$R and CB$_2$R antagonists (SR141716 and SR144528), the relaxant effect was determined to be CB$_1$R-mediated.

In the term human placenta, immunodetectable CB$_1$R was localized to both cytotrophoblasts and syncytiotrophoblasts (32). Acone and colleagues used immunohistochemistry and western blot to evaluate CB$_1$R and FAAH in placentas obtained from laboring and non-laboring subjects (33). They found lower protein density and lesser staining of CB$_1$R in placentas obtained from laboring subjects compared to non-laboring subjects (33). They postulated that AEA up-regulates CB$_1$R to maintain uterine quiescence and that less availability of CB$_1$R is associated with labor. Our group examined CB$_1$R and CRIP1a in human uterine and placental tissue obtained during cesarean deliveries, and we found a significant reduction in CB$_1$R protein in uterine tissue obtained during labor compared to non-labor. Torrela and colleagues evaluated CB$_1$R, CB$_2$R, transient receptor potential vanilloid receptor type 1 (TRPV1), FAAH, NAPE-PLD, monoacylglycerol lipase (MAGL) and diacylglycerol lipase (DAGL) in human placental samples obtained after spontaneous vaginal deliveries using qPCR (36). Compared to samples obtained at 30 weeks gestation (preterm), there was a significant increase in CB$_1$R mRNA at term. The authors also found a significant increase in the NAPE-PLD/FAAH ratio, thus concluding there to be an increase in placental AEA synthesis at term.
Wang and colleagues used a murine model to evaluate the effect of CB1R on parturition (38). They found that CB1R knock-out in mice correlated with the early onset of labor and an early rise in corticotrophin-releasing hormone. In wild type mice, CB1R silencing in late gestation resulted in labor (38). They correlated these findings with serum levels of progesterone and estradiol. CB1R deficient mice were observed to have an early decrease in serum progesterone and increase in serum estradiol levels. Sun and colleagues investigated the effects of sustained AEA signaling in a murine model of lipopolysaccharide (LPS)-induced preterm labor (48, 49). This suggests that we need to re-evaluate findings that attribute physiological processes associated with parturition to PGs, with a new understanding that some of these effects may be due to prostamides. Mitchell and colleagues found that pro-inflammatory cytokines (TNFα, IL-1β) preferentially stimulated PG over prostamide synthesis in human term non-laboring placental choriodecidual tissues (49), and that in amnion tissue explants, IL-1β was particularly efficacious at promoting PGE2 synthesis (50). These results are consistent with findings that amniotic fluid PGs were greater in women in spontaneous labor compared with those delivering without labor (50). In that same study, spontaneous labor amniotic fluid prostamides were lower in women with clinical chorioamnionitis compared with undiseased women. Thus, we can speculate that inflammatory responses to AEA in these tissues is dependent upon FAAH to hydrolyze AEA to arachidonic acid to serve as the substrate for COX-2. In contrast, Fonseca and colleagues noted that AEA promoted apoptosis in cultured rat decidual cells (51). In these cells, AEA stimulated MAPK P38 phosphorylation and disinhibition of the NF-κB to induce COX-2, which subsequently used AEA as a substrate to produce prostamide E2 (43). Prostamide E2, not PGE2, was the COX-2 substrate that initiated the intrinsic apoptosis pathway and reduced cell viability (43).

### PROSTAGLANDINS AND PROSTAMIDES

PG production in term pregnancy and initiation of labor is stimulated by pro-inflammatory cytokines which induce COX-2 and myometrial stretch signals (41, 42). AEA and other CB1R agonists also induced COX-2 expression and PGE2 production in cultured fetal amnion and chorion explants (30, 43). FAAH, expressed in human term placenta (32), metabolizes AEA to produce the arachidonic acid substrate for COX enzymes. Additionally, AEA itself can be oxidized by COX-2 (but not COX1) to PGH2-EA and the subsequent ethanolamides of PGs, referred to as prostamides (44, 45). AEA can also be oxidized by 5-, 12-, or 15-lipoxygenases to produce their respective OH-epoxyeicosatrienoic acid ethanolamide (5,6-EET-EA) (45, 47). It is now recognized that the radioimmunoassays used to quantitate PGs also recognized prostamides, and that the two classes can be distinguished using liquid chromatography-mass spectrometry (48, 49). This suggests that we need to re-evaluate research findings that attribute physiological processes associated with parturition to PGs, with a new understanding that some of these effects may be due to prostamides.

| Author               | ECS Component | Tissue Type | Experimental Method |
|----------------------|---------------|-------------|---------------------|
| Dennedy, et al. (31) | CB1R          | Uterus      | RT-PCR              |
|                      | CB2R          | Uterus      | RT-PCR              |
|                      | FAAH          | Placenta    | IHC                 |
| Park, et al. (32)    | CB1R          | Placenta    | IHC                 |
|                      | FAAH          | Placenta    | IHC                 |
| Acone, et al. (33)   | CB1R          | Placenta    | Western blot, IHC   |
| Fugedi, et al. (34)  | CB1R          | Placenta    | Western blot, IHC   |
|                      | CB2R          | Placenta    | Western blot, IHC   |
|                      | FAAH          | Placenta    | Western blot, IHC   |
| Aban, et al. (35)    | CB1R          | Placenta    | Western blot, IHC   |
|                      | NAPE-PLD      | Placenta    | qPCR                |
|                      | FAAH          | Placenta    | qPCR                |
| Torella, et al. (36) | CB1R          | Placenta    | qPCR                |
|                      | CB2R          | Placenta    | qPCR                |
|                      | NAPE-PLD      | Placenta    | qPCR                |
|                      | FAAH          | Placenta    | qPCR                |
|                      | MAGL          | Placenta    | qPCR                |
|                      | DAGL          | Placenta    | qPCR                |
| Kozakiewicz, et al. (37) | CB1R  | Uterus      | Western blot, qPCR, IHC |
|                      | CB1R          | Placenta    | Western blot, qPCR  |
|                      | CRIP1a        | Placenta    | Western blot, qPCR  |
|                      | CRIP1a        | Placenta    | Western blot, qPCR  |

**TABLE 1 | ECS components in human tissue mid- to late-trimester.**

Dennedy, et al. (31) CB1R, CB2R, FAAH, Western blot, IHC
Acone, et al. (33) CB1R, Placenta, Western blot, IHC
Fugedi, et al. (34) CB1R, CB2R, FAAH, Placenta, Western blot, IHC
Aban, et al. (35) CB1R, NAPE-PLD, FAAH, Placenta, Western blot, IHC
Torella, et al. (36) CB1R, CB2R, NAPE-PLD, FAAH, MAGL, DAGL, Placenta, qPCR
Kozakiewicz, et al. (37) CB1R, CB1R, CRIP1a, Placenta, Western blot, qPCR

PROSTAGLANDINS AND PROSTAMIDES

PG production in term pregnancy and initiation of labor is stimulated by pro-inflammatory cytokines which induce COX-2 and myometrial stretch signals (41, 42). AEA and other CB1R agonists also induced COX-2 expression and PGE2 production in cultured fetal amnion and chorion explants (30, 43). FAAH, expressed in human term placenta (32), metabolizes AEA to produce the arachidonic acid substrate for COX enzymes. Additionally, AEA itself can be oxidized by COX-2 (but not COX1) to PGH2-EA and the subsequent ethanolamides of PGs, referred to as prostamides (44, 45). AEA can also be oxidized by 5-, 12-, or 15-lipoxygenases to produce their respective OH-epoxyeicosatrienoic acid ethanolamide (5,6-EET-EA) (45, 47). It is now recognized that the radioimmunoassays used to quantitate PGs also recognized prostamides, and that the two classes can be distinguished using liquid chromatography-mass spectrometry (48, 49). This suggests that we need to re-evaluate research findings that attribute physiological processes associated with parturition to PGs, with a new understanding that some of these effects may be due to prostamides. Mitchell and colleagues found that pro-inflammatory cytokines (TNFα, IL-1β) preferentially stimulated PG over prostamide synthesis in human term non-laboring placental choriodecidual tissues (49), and that in amnion tissue explants, IL-1β was particularly efficacious at promoting PGE2 synthesis (50). These results are consistent with findings that amniotic fluid PGs were greater in women in spontaneous labor compared with those delivering without labor (50). In that same study, spontaneous labor amniotic fluid prostamides were lower in women with clinical chorioamnionitis compared with undiseased women. Thus, we can speculate that inflammatory responses to AEA in these tissues is dependent upon FAAH to hydrolyze AEA to arachidonic acid to serve as the substrate for COX-2. In contrast, Fonseca and colleagues noted that AEA promoted apoptosis in cultured rat decidual cells (51). In these cells, AEA stimulated MAPK P38 phosphorylation and disinhibition of the NF-κB to induce COX-2, which subsequently used AEA as a substrate to produce prostamide E2 (43). Prostamide E2, not PGE2, was the COX-2 substrate that initiated the intrinsic apoptosis pathway and reduced cell viability (43).
DISCUSSION

The complex mechanisms that normally convert the uterus from a quiescent to an active contractile state remain unclear. The significant decrease in circulating progesterone that initiates labor in most laboratory animals does not occur in humans (52). The available evidence supports that the active contractile transition involves cessation of the inhibitory effects of progesterone and the activation of estrogen production leading to up-regulation of genes and proteins that enhance uterine contractility (52). There are two functionally distinct progesterone receptors, termed progesterone receptor A (PR-A) and progesterone receptor B (PR-B). PR-B signaling functions to promote activation of genes and proteins that enhance uterine relaxation whereas PR-A represses them (53). Sex steroid hormones are known to influence the expression of components of the ECS in various tissues (9, 54, 55). Estradiol influences expression of CB1R and AEA production and degradation in the brain (56). AEA interferes with aromatase transcription and estradiol production in human endometrial stromal cells and human decidual fibroblasts (57). Progesterone increases FAAH activity and expression in human lymphocytes but does not influence CB1R (55). Abnormal fluctuations in serum progesterone and estradiol levels are apparent in CB1R deficient mice (38). As reviewed by Karasu and colleagues, the termed “endocannabinoid-hormone-cytokine network” plays a significant role in implantation and early pregnancy events (10). It is therefore reasonable to theorize that, based on the interconnections between PGs/prostamides, sex steroid hormones and endocannabinoids, the ECS is likely to have a meaningful part in pregnancy maintenance and timing of labor. Studies evaluating the effects of cannabis use in pregnancy have provided mixed results (58, 59). They are limited by confounding factors (polysubstance abuse, tobacco use), the reliance on subject self-reporting, and the perplexity of obtaining a reliable biologic sample for drug testing. Cannabis use in pregnancy has been associated with increased risk of spontaneous preterm birth (60), stillbirth (61), poor fetal growth (59), and adverse neonatal outcomes (62). Data are limited regarding potential effects of marijuana on labor itself. In one of the few studies evaluating labor patterns in marijuana users, Greenland and colleagues found that subjects reporting marijuana use had a higher risk of experiencing prolonged, arrested or precipitous labor (63). Many of the available data regarding cannabis use in pregnancy were collected prior to the decriminalization of marijuana, the introduction of newer methods of cannabis consumption and the introduction of higher potency compounds. Given the increasing prevalence of cannabis use in pregnancy (58, 64), it is imperative to not only evaluate the risks of marijuana use in pregnancy but also to gain a better understanding of the mechanisms by which the ECS contributes to pregnancy maintenance and labor.

AEA is degraded primarily by FAAH to produce arachidonic acid and ethanolamine (16). However, AEA oxidization by COX-2 to PG-ethanolamides (prostamides) (16) highlights a significant overlap between the ECS and PG production. PGs are routinely used in obstetrics for induction of labor and the treatment of postpartum hemorrhage related to uterine atony. Indomethacin, a non-selective COX inhibitor, is one of the recommended first-line tocolytic therapies for preterm labor (18). Indomethacin has been recently identified to be a positive allosteric modulator of CB1R (65). Its modulating effects enhance AEA-dependent binding, β-arrestin 1 recruitment, cAMP inhibition and ERK1/2 phosphorylation (65). Bariani and colleagues found that LPS-induced preterm labor in a murine model correlates with increased CB1R expression and, even without the addition of LPS, administration of AEA resulted in a CB1R-mediated increase in PGF2α (40). In contrast, in a murine model without LPS administration, earlier onset of labor was identified in mice lacking CB1R (38). Although there are limitations to this based on the differences between rodent and human labor, this highlights the possibility that ECS expression may differ in infection-related labor compared to normal term labor.

Although an initial study utilizing AEA and/or PEA as a biomarker in the risk assessment for preterm birth is promising, that evaluation was limited to a population with a higher-risk of preterm birth (21). Additional studies including a more generalized and larger population are needed. The current standard of care involving measurement of cervical length and, in some cases, cervicovaginal fetal fibronectin does not reliably predict preterm birth (18). Additionally, assessment of cervical length requires equipment (ultrasound with transvaginal probe) and personnel with adequate training and who are readily available to perform the exam. A blood test would be more feasible in many situations.

Significant racial disparities exist in the rate of preterm birth (66). This racial disparity persists when evaluating women with similar socioeconomic status (67), leading many to believe that genetic variation may play a role. A cross-sectional study of 667 subjects identified racial differences in CNR1 and FAAH polymorphisms associated with obesity (68). Given the increasing evidence of the ECS involvement in normal and abnormal pregnancy outcomes, genetic variation among the components of the ECS pertaining to abnormal pregnancy outcomes should be explored.

The biology of labor is complex and includes interplay among steroid hormones, cytokines and PGs affecting the maternal-fetal interface (12, 13, 69). There exists a significant overlap between the inflammatory pathway, steroid hormones and endocannabinoids (10). Because the ECS modulates metabolic and inflammatory cell signaling and can modulate cell differentiation, cell proliferation and cell death, it is reasonable to expect that the ECS exerts an influence on the regulation of labor. More research is needed for significant conclusions regarding ECS specific role in pregnancy maintenance and the timing of labor.

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The authors confirm contribution to the manuscript as follows: MK, CG and AH critically reviewed the literature. MK drafted the article. MK, CG and AH reviewed and revised the
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35. Abán C, Leguizamón GF, Cella M, Damiano A, Franchi AM, Farina MG. Differential Expression of Endocannabinoid System in Normal and Preectopic Placentas: Effects on Nitric Oxide Synthesis. *Placenta* (2013) 34(1):67–74. doi: 10.1016/j.placenta.2012.10.009

36. Torella M, Bellini G, Punzo F, Argenziano M, Schiattarella A, Labriola D, et al. Tnf-α Effect on Human Delivery Onset by CB1/TRPV1 Crosstalk: New Insights Into Endocannabinoid Molecular Signaling in Preterm vs. Term Labor. Analysis of the EC/EV Pathway and Predictive Biomarkers for Early Diagnosis of Preterm Delivery. *Minerva Ginecol* (2019) 71(5):359–64. doi: 10.23736/S0026-4784.19.04405-8

37. Kozakiewicz ML, Zhang J, Leone-Kabler S, Yamaleyeva LM, McDonald AG, et al. Down-Regulation of Anandamide Hydrolase in Mouse Uterus by Sex Hormones. *Eur J Biochem* (2000) 267(10):2991–7. doi: 10.1046/j.1432-0486.2000.01316.x

38. Kozakiewicz ML, Valensine H, Bari M, Lazzarin N, Romanini C, Finazzi-Agrò A. Progestrone Up-Regulates Anandamide Hydrolase in Human Lymphocytes: Role of Cytokines and Implications for Fertility. *J Immunol* (2001) 166(12):7183–9. doi: 10.4049/jimmunol.166.12.7183

39. González S, Bisogno T, Wengen T, Manzanares J, Milone A, Berrendero F, et al. Sex Steroid Influence on Cannabinoid CB(1) Receptor mRNA and Endocannabinoid Levels in the Anterior Pituitary Gland. *Biochem Biophys Res Commun* (2000) 270(1):260–6. doi: 10.1006/bbrc.2000.2406

40. Almada M, Oliveira A, Amaral C, Fernandes PA, Ramos MJ, Fonseca B, et al. Anandamide Target Aromatase: A Breakthrough on Human Decidualization. *Biochem Biophys Acta Mol Cell Biol Lipids* (2019) 1864(12):158512. doi: 10.1016/j.bbalip.2019.08.008

41. Conner SN, Bedell V, Lippedy K, Macrones GA, Cahill AG, Tuuli MG. Maternal Marijuana Use and Adverse Neonatal Outcomes: A Systematic Review and Meta-Analysis. *Obstet Gynecol* (2016) 128(4):713–23. doi: 10.1097/AOG.0000000000001649

42. Lernaat SG, Dekker GA, McCowan LM, Kenny LC, Myers IE, Simpson NA, et al. Maternal Marijuana Use Has Independent Effects on Risk for Spontaneous Preterm Birth But Not Other Common Late Pregnancy Complications. *Reprod Toxicol* (2016) 62:77–86. doi: 10.1016/j.reprotox.2016.04.021

43. Warner MV, Silver RM, Rowland Hogue CJ, Willinger M, Parker CB, Thorsten VR, et al. Association Between Stillbirth and Illicit Drug Use and Smoking. *Obstet Gynecol* (2014) 123(1):113–25. doi: 10.1097/PGO.0000000000001052

44. Metz TD, Bahlhour AA, Hogue CJ, Goldenberg RL, Dudley DJ, Warner MV, et al. Maternal Marijuana Use, Adverse Pregnancy Outcomes, and Neonatal Morbidity. *Am J Obstet Gynecol* (2017) 217(4):478e1–8. doi: 10.1016/j.ajog.2017.05.050

45. Greenland S, Staisch KJ, Brown N, Gross SJ. The Effects of Marijuana Use During Pregnancy. I. A Preliminary Epidemiologic Study. *Am J Obstet Gynecol* (2004) 181(4):408–13. doi: 10.1016/S0002-9378(04)00282-5

46. Brown QL, Sarvet AL, Shmulewitz D, Martins SS, Wall MM, Hasin DS. Trends in Marijuana Use Among Pregnant and Nonpregnant Reproductive-Aged Women, 2002–2014. *JAMA* (2017) 317(2):207–9. doi: 10.1001/jama.2016.17383

47. Laprairie RB, Mohamed KA, Zaggoz A, Kelly MEM, Stevenson LA, Pertwee R, et al. Indomethacin Enhances Type 1 Cannabinoid Receptor Signaling. *Prostaglandins Leukot Essent Fatty Acids* (2016) 107:185–92. doi: 10.1016/j.prostaglandins.2016.07.004

48. Almada M, Correia-da-Silva G, Teixeira NA. Anandamide-Induced Cell Death: Dual Effects in Primary Rat Decidual Cell Cultures. *Placenta* (2009) 30(8):686–92. doi: 10.1016/j.placenta.2009.05.012

49. Norwitz ER, Mahendroo M, Lye SJ. CreaSy and Resnik's Maternal-Fetal Medicine: Principles and Practice. 8th Edition. R Resnik, CJ Lockwood, TR Moore, MF Greene, JA Copel, RM Silver, editors. Philadelphia, PA: Elsevier (2019).

50. Peters GA, Yi L, Skomorovská-Prokvotil Y, Patel B, Amini P, Tan H, et al. Inflammatory Stimuli Increase Progestrone Receptor-A Stability and Transrepressive Activity in Myometrial Cells. *Endocrinology* (2017) 158(1):158–69. doi: 10.1210/endo.2016-1537

51. MacCarrone M, De Felici M, Bari M, Klinger F, Siracusa G, Finazzi-Agrò A. Down-Regulation of Anandamide Hydrolase in Mouse Uterus by Sex Hormones. *Eur J Biochem* (2000) 270(10):2991–7. doi: 10.1046/j.1432-0486.2000.01316.x

52. MacCarrone M, Valensine H, Bari M, Lazzarin N, Romanini C, Finazzi-Agrò A. Progestrone Up-Regulates Anandamide Hydrolase in Human Lymphocytes: Role of Cytokines and Implications for Fertility. *J Immunol* (2001) 166(12):7183–9. doi: 10.4049/jimmunol.166.12.7183

53. Kozakiewicz ML, Zhang J, Leone-Kabler S, Yamaleyeva LM, McDonald AG, et al. Down-Regulation of Anandamide Hydrolase in Mouse Uterus by Sex Hormones. *Eur J Biochem* (2000) 267(10):2991–7. doi: 10.1046/j.1432-0486.2000.01316.x

54. MacCarrone M, De Felici M, Bari M, Klinger F, Siracusa G, Finazzi-Agrò A. Down-Regulation of Anandamide Hydrolase in Mouse Uterus by Sex Hormones. *Eur J Biochem* (2000) 267(10):2991–7. doi: 10.1046/j.1432-0486.2000.01316.x

55. Almada M, Oliveira A, Amaral C, Fernandes PA, Ramos MJ, Fonseca B, et al. Anandamide Targets Aromatase: A Breakthrough on Human Decidualization. *Biochem Biophys Acta Mol Cell Biol Lipids* (2019) 1864(12):158512. doi: 10.1016/j.bbalip.2019.08.008

Conflict of Interest: The author CG is the Chief Medical Officer of Nixxi (https://nixxihealth.com), a company developing preterm birth prediction tools. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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