The Endocannabinoid System - The Prediction of Spontaneous Preterm Birth in High-Risk Women: Protocol of a Study

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Summary

Spontaneous preterm birth (sPTB) is a major cause of perinatal morbidity and mortality, even in developed countries. Prediction of sPTB is therefore a valuable tool to reduce the associated risks. The current standard for the prediction of sPTB consists, in addition to anamnestic data, of previous sPTB and previous second trimester miscarriage, measurement of cervical length by transvaginal ultrasound (TVU CL) together with assessment of fetal fibronectin levels in cervicovaginal fluid. Other evaluation parameters, such as the level of endocannabinoids in the pregnant woman's blood, could increase the sensitivity of this management. Endocannabinoids (eCBs) are a part of the endocannabinoid system (ECS); out of them anandamide (arachidonoyl-ethanolamide, AEA), in particular, plays an important role in the regulation of pregnancy and childbirth. We present the protocol for an open, non-randomized study to evaluate concentrations of AEA and other endocannabinoids: 2-linoleoylglycerol (2-AG), 2-linoleoylglycerol (2-LG), 2-oleoylglycerol (2-OG), and 2-arachidonoyldopamine (2-ADOPA or also NADA) in the blood of pregnant women as potential predictors of sPTB. In a total of 230 women with a history of sPTB or miscarriage, eCBs levels between 22 and 28 weeks of gestation will be assessed from maternal blood, in addition to the standard procedure. The aim of the study is to determine the relationship between blood concentrations of the endocanna-binoids tested and the risk of sPTB. The results of this study will describe the prognostic significance of maternal blood eCBs levels for sPTB, and could subsequently enable improved screening programs for early identification of sPTB.

Keywords

Anandamide (AEA) • Endocannabinoids • Endocannabinoid system • 2-arachidonoylglycerol (2-AG) • 2-linoleoylglycerol (2-LG) • 2-oleoylglycerol (2-OG) • 2-arachidonoyldopamine (2-ADOPA • NADA) • Predicting preterm birth • Spontaneous preterm birth (sPTB) • Preterm labor • Prediction

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Introduction

Spontaneous preterm birth (sPTB) is one of the leading causes of perinatal morbidity and mortality. Despite modern advances in obstetrics and neonatology, the number of preterm births has not been significantly reduced everywhere in the world. Its incidence is as high as 18 % in some countries [1]. Although it is 6.98 % in the conditions of the Czech Republic [2], this still has to be considered an incidence that must be reduced. This is due to the extensive societal impact of sPTB due to increased infant mortality, the lifetime risk of various health complications and, of course, the significant economic burden.

Many risk factors have been described concerning sPTB, yet the ability to accurately predict the timing of human birth, whether at term or preterm, remains elusive. The sPTB, which results from multifactorial effects on uterine muscle contractility, is usually only highlighted by its clinical manifestations.

A large variety of biochemical markers have been studied as a potential cause for or consequence of the pathophysiological mechanisms leading to human reproductive pathology [3]. Consequently, endocannabinoid metabolism represents a novel and promising target, as this system plays an important role in the development and implantation of the blastocyst, embryo, placentation, gestational length and human birth. Endocannabinoid metabolism is widely intertwined with prostaglandin metabolism and its effect on uterine muscle contraction. The evaluation of endocannabinoid levels in the maternal serum could identify the family of asymptomatic women at high risk of sPTB. Consequently, such additional test would be valuable for the prevention or early treatment/elimination of this serious pathology of the human reproductive process [4].

Endocannabinoids (eCBs) are signaling molecules that are naturally produced by the human or animal body. The endocannabinoid system (ECS) is based upon eicosanoid derivatives that promote the cellular return to homeostasis in multiple organ systems and modulates smooth muscle function, metabolism of tissues, and immune function [4]. The ECS is associated with several physiological processes, including reproduction [5]. The ECS consists of cannabinoid receptors (CB), cannabinoid ligands i.e., endocannabinoids, membrane transporters, and the metabolic enzymes that modulate endocannabinoid synthesis and breakdown [6].

The ECS is significantly involved in physiological processes in the female reproductive system, where it plays a major role in embryo development, implantation, and placentation. Together with sex hormones and prostaglandins, eCBs also play an important role in the timing of childbirth [7]. The published results suggest that eCBs may be a sensitive predictor of sPTB [8]. However, the physiological and pathological relationship of eCBs to sPTB is still poorly understood. For example, specific eCBs essential for the prediction of sPTB have not been identified. Moreover, the optimal schedule for the relevant tests during pregnancy has not yet been determined. Finally, the suitability of blood levels or non-invasive testing of endocannabinoid levels from saliva remains to be established.

The review [9] showed that ECS plays a significant role in embryo development, transport and implantation as well as in placentation. It consists of numerous endogenous ligands. However, only anandamide (AEA) and 2-arachidonoylglycerol (2-AG) represent relevant markers related to pregnancy. The study also showed that AEA plays a regulatory role in pregnancy maintenance and the timing of labor in addition to early pregnancy events. Interestingly, the activity of AEA depends on its metabolic pathway and the enzymatic activity that ensures its conversion. Ultimately, changes in AEA concentration lead to increased production of prostaglandins or prostamides, with inverse effects on pregnancy. The abuse of exogenous cannabinoids in pregnancy has a substantial impact on the unborn child in many ways and may result in detrimental effects including preterm birth.

Correlations between endocannabinoid levels and sPTB risk have been published recently [10]. It has been shown that women between 24^{+0} and 34^{+0} weeks of gestation at high risk of PTB have a positive correlation between endocannabinoid levels and PTB. AEA and N-palmitoylethanolamide (PEA) levels above 1.095 nM and 17.5 nM in plasma, respectively, were predictors of sPTB. In contrast, AEA level was simultaneously a predictor of gestational age at delivery and the remaining time to delivery. The predictive value appeared to be higher than previously used prediction methods, which could further improve the prevention of sPTB.

According to literature, the endocannabinoid levels are dependent on circadian rhythm and in the case of AEA, reach two peaks within 24 hours (at 2 am and around 3 pm) with a nadir around 10 am [11]. In contrast, 2-AG and 2-OG levels reached nadir from 2 am to 4 am.

Then, the concentrations gradually increased and reached approximately triple (2-AG) or double (2-OG) values, respectively, around midday [12]. Therefore, in this study, the sampling of biological material is scheduled for the AEA nadir period, 9-12 am.

Improved prediction of sPTB is associated with a significant reduction in hospital stay for mothers, as well as a reduction in healthcare costs for treatments, medical procedures. Most importantly, the improved prediction of sPTB would contribute significantly to the reduction in neonatal mortality and morbidity [13]. Finally, artificial intelligence possesses a great potential to predict sPTB correctly [14] however, relevant and strong data from clinical studies need to be generated. Altogether, we believe that the proposed study could contribute significantly to the scientific background of sPTB research.

Research objectives

The findings of the main objective (see below) could usefully complement the standard screening program with an additional independent predictor of sPTB and thus increase the sensitivity of screening.

The aim of the study is to assess changes in plasma levels of components of the endocannabinoid system between 24 and 28 weeks of gestation and to evaluate their relationship to the onset of labor. It can be expected that changes in peripheral blood endocannabinoid levels could be used as an objective parameter to predict sPTB. Participation in the project will be offered to women between 24 and 28 weeks of pregnancy with a burdened obstetric history. Pregnant women will have venous blood drawn and analyzed at the Institute of Physiology of the Czech Academy of Sciences. The standard prediction of sPTB consists of the quantitative determination of fetal fibronectin (qfFN) and transvaginal ultrasound cervical length (TVU CL) in addition to the obstetric history (e.g. sPTB, miscarriage after 16^{+0} weeks of pregnancy) [15].

In addition to the standard predictors of sPTB and AEA levels, the study also focuses on determining the levels of four other endocannabinoids, namely 2-AG, 2-LG, 2-OG and 2-ADOPA. The correlation of the levels of the individual components of ECS has not been studied before in such a range of endocannabinoids.

We hypothesize that changes in endocannabinoid levels from peripheral venous maternal blood can be used as a tool for more accurate positive prediction of sPTB.

The standard prediction of sPTB in high-risk pregnancies consists of the quantitative determination of fetal fibronectin (qfFN) and transvaginal ultrasound cervical length (TVU CL) in addition to the obstetric history.

Methods and analysis

Study design

The study will be a prospective observational single-center study.

Study sites

The study will be conducted at a single center, i.e., at the Department of Gynecology, Obstetrics and Neonatology of the 1st Faculty of Medicine, Charles University and General University Hospital in Prague, in collaboration with the Institute of Physiology of the Czech Academy of Sciences, where examinations of endocannabinoid levels will take place.

Training of investigators

All doctors participating in the study will receive training in proper clinical practice, or will be certified in this area. At the same time, they will be thoroughly familiarized with the study protocol and, to the necessary extent, also with the procedures of proper laboratory practice.

Participants

Inclusion criteria: Women with singleton pregnancies with a history of sPTB up to 34 weeks of pregnancy or women with miscarriage from 16 weeks of pregnancy will be included in the study. Inclusion criteria will be always negative pregnancy screening for chromosomal aberrations in the first trimester (combination of ultrasound and biochemical examination), with intact membranes and physiological findings on ultrasound examination – detailed fetal morphology at 20 – 22 weeks of pregnancy. Further, that the collection/homogeneity of pregnant women applies to the whole Czech Republic. Participation in the project will be offered to women coming to the Department of Gynecology, Obstetrics and Neonatology of the 1st Faculty of Medicine, Charles University and General University Hospital in Prague. It will be offered to women registered at the clinic for examination due to an imminent spontaneous sPTB or meeting the inclusion

criteria of the project. Participation in the study will be completely voluntary; the women will have the right to withdraw from the study at any time.

Exclusion criteria: Congenital developmental defect, medical abortion/premature birth, multiple pregnancy, absence of informed consent and age < 18 years.

Withdrawal criteria: An investigator may terminate an individual's participation in the research study to:

a) protect the participant from excessive risk or risk with a demonstrated lack of benefits (e.g., a participant experiences a serious side-effect without the anticipated therapeutic effects), or

b) maintain the integrity of the data (e.g., a participant is not following study procedures or may be deliberately providing false information).

Sample size

Due to the nature of the study, 230 women will be included in the study. A sample size calculation was performed. With a confidence interval of 95 %, margin of error of 5 %, a population proportion of 8 % (estimated proportion of preterm birth in the facility), and a total population size of 4800 (estimated number of births in the facility during one year), the calculated sample size is 111. Thus the 230 women we expected to include into the study in the might be consider as sufficient sample.

Procedures

As a result, plasma levels of individual components of the endocannabinoid system will be determined in women at high risk of sPTB. The results obtained will be related to the clinical outcome of pregnancy. Multiregression analysis of the data obtained will identify individual components of the endocannabinoid system in relation to delivery within one, two and four weeks of examination and sPTB before 34 and 37 weeks of gestation. The sensitivity and specificity of each component in predicting sPTB will be determined. Subsequently, the results will be compared with previously used predictors of sPTB.

Each woman enrolled in the study will undergo a clinical examination and risk quantification sPTB are the standard procedures used by the study site. The standard prediction of sPTB consists of the quantitative determination of (qfFN) and (TVU CL), in addition to the obstetric history (e.g., sPTB, miscarriage after 16 weeks of gestation). The examination will take place between 24 to 28 weeks of gestation. Each woman will undergo clinical and, if necessary, other examinations as appropriate to the conditions of routine clinical practice.

Except for these examinations, no interventional examinations will be performed beyond the scope of normal obstetric practice.

Data collection and follow-up

The women will have 5 ml of venous blood drawn and centrifuged immediately after collection. Collection of biological samples is planned during the AEA nadir period, between 9 – 12 am, to account for circadian rhythm influences. The plasma obtained will be immediately frozen at –80 °C. After transport to the Institute of Physiology of the Czech Academy of Sciences, the samples will be subjected to metabolomic analysis using a high-resolution mass spectrometer (Thermo Q Exactive Plus) with a liquid chromatograph - Thermo Vanquish [16].

The method of data recording: electronic eCRF without possibility of correction by the investigator. The intended duration of the project is 2 years maximum. We plan to enroll the sample of patients in the trial during the first 12 months. In the next four months, the analysis of the blood samples and the statistical processing of the results will be carried out. During the last project year, the results will be published in peer-reviewed journals and presented at professional conferences in the Czech Republic and abroad.

The relationship between plasma levels of individual endocannabinoids and time of onset of labor will be investigated.

The following markers will be investigated: arachidonic acid (AA) levels, anandamide (AEA) levels, 2-arachidonoylglycerol (2-AG) levels, 2-linoleoyl-glycerol (2-LG) levels, 2-oleoylglycerol (2-OG) levels, 2-arachidonoyldopamine (2-ADOPA) levels, fetal fibronectin (fFN) levels in cervicovaginal fluid.

Targeted analysis of lipid mediators

A volume of 675 µl of methanol will be added to a 2 ml Eppendorf tube (microtube) on ice, then 10 µl of an internal standard mixture (arachidonoyl ethanolamide-d4 and 2-arachidonoyl glycerol-d5; Cayman Chemical) in methanol will be added followed by 1 ml of plasma. The tube will be vortexed (10 s) and centrifuged (10 min, 12,000´g, 4 °C). Supernatant from the 2 ml Eppendorf tube will be added to a 6 ml glass tube on ice containing 2.825 ml of cold water. After vortexing (5 s), the extract

will be applied into the solid-phase extraction (SPE) column (Strata-X, 200 mg/3 ml; Phenomenex) previously washed out with 6 ml of ethyl acetate (removing contaminants), 6 ml of methanol (sorbent activation), and 6 ml of water (separation environment). After passing the sample through the SPE using a vacuum, the column will be washed with 6 ml of cold water till dry. Meanwhile, the new collecting 6 ml tube containing 10 µl of 30 % glycerol in methanol will be prepared to capture the analytes after the drying step. The analytes will be eluted with 6 ml of ethyl acetate, then will be dried in a Speedvac until the final drop of glycerol. The extract will be then dissolved in 200 µl of a resuspension mixture (30 % acetonitrile, 60 % water, and 10 % methanol) and transferred to an LC vial with a microinsert.

Purified extracts will be analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) operated in multiple reaction monitoring (MRM). The LC-MS/MS system consists of an Ultimate 3000 RSLC (Dionex/Thermo) coupled with a QTRAP 5500 (SCIEX). The Kinetex C18 column (150 mm × 2.1 mm i.d.; 2.6 µm particle size) will be used to separate target metabolites. Separation will be carried out at a flow rate of 0.3 ml/min with the column at 50 °C. The mobile phase included (A) water/acetonitrile (7:3) with 0.02 % acetic acid, and (B) acetonitrile/isopropanol (1:1). Separation will be achieved through the following gradient: 0–0.53 min 0 % (A); 0.53–1.73 min from 0 % to 25 % (B); 1.73 – 6.53 min from 25 % to 45 % (B), 6.53–7.73 min from 45 % to 60 % (B), 7.73–10.73 min from 60 % to 75 % (B), 10.73 – 11.03 min from 75 % to 90 % (B), 11.03–11.93 min at 90 % (B); 11.93–12.5 min from 90 % to 100 % (B), 12.5–13 min from 100 % to 0 % (B); 13–16 min at 0 % (B) +2 min preinjection steps.

The injection volume will be 10 µl, and the sample temperature will be maintained at 4 °C.

Detailed information on MRM transitions can be found elsewhere [16]. PeakView software (SCIEX) will be used for data processing. For anandamide (AEA) and 1- and 2-AG, data will be reported in pmol/ml plasma, while the rest of the lipid mediators will be reported in signal intensity of the detector.

Quality control will be ensured by (i) randomization of the samples within the sequence, (ii) regular insertion of quality control (QC) pool samples at the beginning, end, and between every ten actual samples, (iii) analysis of method blanks, and (iv) control of the chromatographic peak shape, retention time, and intensity of internal standards.

Planned results analysis

Statistical analysis will be performed using NCSS 2022 Update statistical software. A descriptive analysis of the characteristics of the study population will be performed. Furthermore, the demographic characteristics of the subgroup of women who experienced sPTB and women who delivered at term will be compared. Endocannabinoids levels will be reported in µmol/l (µM), nmol/l (nM) or pmol/l (pM), respectively. The χ-squared test and Fisher's exact test will be used to assess differences in endocanabiods levels between subgroups. ANOVA test will be used to compare continuous variables between the two groups. Results of continuous variables will be reported as means and standard deviations, or as medians with 25 and 75 percentiles. A p-value < 0.05 will be considered statistically significant.

Patient and public involvement

Ethics and dissemination

The study was approved by the Ethics Committee of the General University Hospital in Prague on 19th July 2018, No. 1109/18 S, observational prospective cohort study. Due to the nature of the study, approval by the national drug regulatory authority (State Institute for Drug Control) was not required.

Implications for interventions and future policy

Prediction of the risk of sPTB is standardly performed in all women with a history of obstetric complications who are admitted to the General University Hospital in Prague. The reasons have already been mentioned and consist mainly in an increase in neonatal mortality and the development of late complications in some preterm neonates [17,18,19,20]. Our study aims to identify another predictor that would be independent of the predictors currently used in standard management [21].

The relationship between AEA levels and pregnancy was described already in 2004 [22]. Other research is focused on the correlation between AEA levels and time to delivery [23]. The role of the endocannabinoid system in pregnancy, at delivery and in sPTB has recently been the subject of a review article [4].

Increased sensitivity and specificity of prediction are associated with a higher probability of positive

detections and a reduction in false positives. As already mentioned, at least two endocannabinoids (AEA and 2-AGPEA) can be considered as predictors of sPTB with a high sensitivity and specificity. It has not yet been definitively confirmed at what time of pregnancy it is appropriate to perform biological sampling, whether it is necessary to detect endocannabinoid concentrations in blood, or whether their determination in saliva is sufficient to avoid potentially redundant interventional testing, and whether AEA are the only predictors.

The expected findings could improve screening management with an additional independent predictor of sPTB.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The study was approved by the ethics committee of the General University Hospital in Prague on 19th July 2018, No. 1109/18 S, observational prospective cohort study. Due to the nature of the study, approval by the national drug regulatory authority (State Institute for Drug Control) was not required. All women provided written an informed consent before participation.

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References

1. WHO Preterm birth. Acessed 30. 8. 2023 https://www.who.int/news-room/fact-sheets/detail/preterm-birth
2. Velebil P. 2022. Results of perinatal care in the Czech Republic in 2019. Accessed 30. 8. 2023. https://www.mzcr.cz/wp-content/uploads/2020/07/V %C3 %BDsledky-perinat %C3 %A1ln %C3 %AD-p %C3 %A9 %C4 %8De-za-rok-2019.pdf
3. Aung MT, Yu Y, Ferguson KK, et al. Prediction and associations of preterm birth and its subtypes with eicosanoid enzymatic pathways and inflammatory markers. Scientific Reports 2019;9(1). https://doi.org/10.1038/s41598-019-53448-z
4. Kozakiewicz ML, Grotegut CA, Howlett AC. Endocannabinoid system in pregnancy maintenance and labor: A mini-review. Front Endocrinol 2021;12. https://doi.org/10.3389/fendo.2021.699951
5. Ezechukwu HC, Diya CA, Shrestha N, Hryciw DH. Role for endocannabinoids in early pregnancy: Recent advances and the effects of cannabis use. Am J Physiol-Endocrinol Metabol 2020;319: E557-E561. https://doi.org/10.1152/ajpendo.00210.2020
6. Martínez-Peña AA, Perono GA, Gritis SA, et al. The impact of early life exposure to cannabis: The role of the endocannabinoid system. Int J Mol Sci 2021;22:8576. https://doi.org/10.3390/ijms22168576
7. Maia J, Fonseca B, Teixeira N, Correia-da-Silva G. The fundamental role of the endocannabinoid system in endometrium and placenta: Implications in pathophysiological aspects of uterine and pregnancy disorders. Human Reproduction Update. 2020;26:586-602. https://doi.org/10.1093/humupd/dmaa005
8. Bachkangi P, Taylor AH, Bari M, Maccarrone M, Konje JC. Prediction of preterm labour from a single blood test: The role of the endocannabinoid system in predicting preterm birth in high-risk women. Eur J Obst Gynecol Reprod Biol 2019;243:1-6. https://doi.org/10.1016/j.ejogrb.2019.09.029
9. Laštůvka Z, Suchopár J, Mašková S, Alblová M, Pařízek A. The role of endocannabinoids in pregnancy. Česká gynekologie 2021;86:349-354. https://doi.org/10.48095/cccg2021349
10. Bachkangi, P. The Role of the Endocannabinoid System in Preterm Birth. University of Leicester, 2018. Accessed 30. 8. 2023. https://hdl.handle.net/2381/42773

11. Hanlon EC. Impact of circadian rhythmicity and sleep restriction on circulating endocannabinoid (eCB) N-arachidonoylethanolamine (anandamide). Psychoneuroendocrinology 2020, 111, 104471. https://doi.org/10.1016/j.psyneuen.2019.104471
12. Hanlon EC, Tasali E, Leproult R, Stuhr KL, Doncheck E, de Wit H, Hillard CJ, Van Cauter E. Circadian rhythm of circulating levels of the endocannabinoid 2-arachidonoylglycerol. J Clin Endocrinol Metab 2015;100:220-226. https://doi.org/10.1210/jc.2014-3455
13. Burchard J, Markenson GR, Saade GR, et al. Clinical and economic evaluation of a proteomic biomarker preterm birth risk predictor: Cost-effectiveness modeling of prenatal interventions applied to predicted higher-risk pregnancies within a large and diverse cohort. J Med Econom 2022;25:1255-1266. https://doi.org/10.1080/13696998.2022.2147771
14. Wong K, Tessema GA, Chai K, Pereira G. Development of prognostic model for preterm birth using machine learning in a population-based cohort of Western Australia births between 1980 and 2015. Scientific Reports 2022;12. https://doi.org/10.1038/s41598-022-23782-w
15. Ruma MS, Banker WM. Availability and use of fetal fibronectin testing and transvaginal ultrasound for preterm labor evaluation in the United States. The Journal of Maternal-Fetal & Neonatal Medicine 2021;35:8586-8593. https://doi.org/10.1080/14767058.2021.1989403
16. Dumlao DS, Buczynski MW, Norris PC, Harkewicz R, Dennis EA. High-throughput lipidomic analysis of fatty acid derived eicosanoids and N-acylethanolamines. Biochim Biophys Acta 2011;1811:724-736. https://doi.org/10.1016/j.bbalip.2011.06.005
17. Adamcová K, Kolátorová L, Škodová T, Šimková M, Pařízek A, Stárka L, Dušková M. Steroid hormone levels in the peripartum period - differences caused by fetal sex and delivery type. Physiol Res 2018;67(Suppl 3):S489-S497. https://doi.org/10.33549/physiolres.934019
18. Pašková A, Pařízek A, Hill M, Velíková M, Kubátová J, Dušková M, Adamcová K, Koucký M, Simjak P, Černý A, Stárka L. Steroid metabolome in the umbilical cord: is it necessary to differentiate between arterial and venous blood? Physiol Res. 2014;63:115-126. https://doi.org/10.33549/physiolres.932624
19. Pařízek A, Mikešová M, Jirák R, Hill M, Koucký M, Pašková A, Velíková M, Adamcová K, Šrámková M, Jandíková H, Dušková M, Stárka L. Steroid hormones in the development of postpartum depression. Physiol Res 2014;63(Suppl 2):S277-S282. https://doi.org/10.33549/physiolres.932788
20. Adamcová K, Kolátorová L, Chlupáčová T, Šimková M, Jandíková H, Pařízek A, Stárka L, Dušková M. Changes to fetal steroidogenesis caused by maternal smoking. Physiol Res 2017;66(Suppl 3):S375-S386. https://doi.org/10.33549/physiolres.933728
21. Bandres-Meriz J, Kunz C, Havelund JF, et al. Distinct maternal metabolites are associated with obesity and glucose-insulin axis in the first trimester of pregnancy. Int J Obesity 2023;47:529-537. https://doi.org/10.1038/s41366-023-01295-4
22. Habayeb OM, Taylor AH, Evans MD, et al. Plasma levels of the endocannabinoid anandamide in women-a potential role in pregnancy maintenance and labor? J Clin Endocrinol Metab 2004;89:5482-5487. https://doi.org/10.1210/jc.2004-0681
23. Nallendran V, Lam P, Marczylo T, et al. The plasma levels of the endocannabinoid, anandamide, increase with the induction of labour. BJOG:J Int Obster Gynaecol 2010;117:863-869. https://doi.org/10.1111/j.1471-0528.2010.02555.x