Pathophysiology of Preterm Labour

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Abstract: A specific pathogenic process of premature delivery represents the inflammation. Birth canal infections seem to play a key role in the ethiopathogenesis of premature delivery; the related biochemical changes significantly affect perinatal morbidity and mortality. Other potential causes, particularly hormone metabolism disorders or uteroplacental ischaemia have been intensively studied. This process is related both to the mother and fetus. Fetal inflammatory response (FIRS) – can occur without maternal response – and it is related to a significant increase in perinatal morbidity. FIRS has definite laboratory, histological and clinical criteria. Effective primary prevention of premature delivery does not exist at present. The sensitivity and specificity of so far used laboratory markers is low. Thus, the research is focused on finding new inflammation markers allowing the early identification of pregnant women at a high risk of premature delivery and fetal inflammation. The screening of women at a high risk by means of new laboratory and ultrasound tests belongs to the most important steps in secondary and tertiary prevention of premature delivery. Intensive research of potential trigger mechanisms has been carried out, including a variety of gene types, which are potentially related to the process of premature delivery.

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Introduction

A rising number of information concerning pathophysiological mechanisms of premature delivery enables new diagnostic and therapeutic procedures to be included into the management of delivery. At present, the ethiopathogenesis of premature delivery is assumed to include the following processes: infection, uteroplacental ischaemia, hormone metabolism disorders, particularly those of gestagens and CRH, impaired maternal tolerance of fetus (theory "fetus as an allograft"), allergy, excessive uterine distension, cervical incompetence. The basic pathogenic process of premature delivery is inflammation. Numerous works have been published, giving evidence of the relation between the above mentioned mechanisms and triggering of the inflammatory process/premature delivery.

The present nomenclature describes premature delivery syndrome. Its ethiopathogenesis might include several causes with possible mutual combination assumed [1].

The abovementioned causes, i.e. infection, uteroplacental ischaemia and hormone metabolism disorders, mainly those of gestagens. The most significant cause of premature delivery is infection – the data available, however, suggest that it is not the infection agent itself which triggers the process of premature delivery; in addition, the presence of genetic predisposition (gene interaction with the environment) is necessary [2].

Pathophysiology of premature delivery

Infection and premature delivery

The action of micro-organisms results in the development of fetal and/or maternal inflammatory response – chorionamnioitis, funisitis and particularly Fetal Inflammatory Response Syndrome – FIRS [3].

These symptoms are recognised as intraamnial inflammation. There is no exact Czech equivalent to the term FIRS so far, the common term is fetal inflammation with an analogous term for adults – SIRS (Systemic Inflammatory Response Syndrome). Fetuses with fetal inflammatory response have higher perinatal morbidity, particularly due to severe stage (III. and IV.) intraventricular haemorrhage (IVH), periventricular leukomalacia, necrous enterocolitis, bronchopulmonary dysplasia, myocardial dysfunction and neonatal sepsis with more frequent incidence of premature delivery, compared with fetuses without inflammatory response. The elevation of interleukin-6 in umbilical blood is post partum followed by significantly higher incidence of funisitis (umbilical inflammation); the inflammation is considered as a histological symptom of FIRS. At present, the basic criteria include the elevation of cytokine levels (particularly interleukin-6), elevated levels of immature forms of neutrophilic granulocytes, elevated CRP in umbilical blood sampled after the delivery and histological, (funisitis, chorionamnioitis) and clinical correlates mentioned above. In an experiment, a diagnostic criterion is the elevation of interleukin-6 in umbilical blood sampled during cordocentesis [4].

The pathogenesis of premature delivery is particularly connected with cytokines, matrixmetalloproteinases and prostaglandins [5, 6]. The trigger effect is mainly seen in pattern recognition receptors – PRR. These receptors have an ability to identify certain molecular structures, common in the majority of micro-organisms. Additionally, they however bind "dangerous signals" from damaged tissue – oxidative stress products (possible non-infectious pathway of premature delivery). The most important group is represented by Toll-like receptors, TLR. The binding of ligand on PRR/TLR receptors results in the activation of nuclear kappa B factor, whose stimulation is connected with the stimulation of cytokine, matrixmetalloproteinase and growth factors genes transcription [7]. Some of the cytokines have pro- or anti-inflammatory effects, e.g. interleukin-6 and -8 or interleukin-10, respectively. In some interleukins, their role is changed dynamically during the inflammation, e.g. in TGF-transforming growth factor. Interleukin-10 is considered as one of the key factors for pregnancy "maintenance" [8]. TGF-beta1 represents one of the cytokines of the initiation phase of inflammation and is studied in correlation with the condition of fetus as an allograft [9].

The basic clinical symptoms of birth canal inflammation are uterine contractions and cervical changes resulting in premature delivery and premature rupture of membranes (PROM). The particular symptoms might affect each other. All clinical signs might be connected with fetal inflammation – FIRS. The tools for prenatal diagnostics of intraamnial infection/inflammation are limited. There is no primary prevention of premature birth so far. The secondary prevention is carried out by defining the risk group of pregnant women. With certain precautions, early identification of the risk group might delay the premature delivery.

In the majority of patients, clinical symptoms of intraamnial inflammation are seen as late as in advanced stages; our aim is early diagnosis of the inflammation. In suspect or already existing premature delivery, particularly in Premature Rupture of Membranes (PROM) it is necessary to eliminate intraamnial infection/inflammation at the admission to the delivery room. The sensitivity of the present tests, i.e. neutrophil granulocyte differential count and the determination of CRP in maternal serum, is relatively low; in addition, these tests mainly give a picture of maternal compartment. Various studies suggest that some women develop fetal infection/inflammation (FIRS) even without the presence of maternal inflammatory response, e.g. to microbial colonisation [10].

The interpretation of results of the cultivation from birth canal is complicated – is this birth canal microbial colonisation or an intraamnial inflammation agent? Birth canal microbial colonisation is not necessarily related to the risk of premature delivery; however, it might enhance the perinatal morbidity and mortality. The routine treatment of asymptomatic microbial invasion in birth canal does not result in the reduction of the incidence of premature delivery [11].

While in already diagnosed PROM it is impossible to give a reliable evidence of a causal relationship between the presence of bacteria in birth canal and the

process of premature delivery or the presence of common birth canal colonisation, ultrasound examination is helpful in prenatal diagnostics.

Ultrasound cervical examination with a vaginal probe – cervicometry – might help distinguish between amniotic cavity, chorion or placenta infections and common colonisation. Microbial invasion of amniotic cavity (MIAC) is connected with qualitative (funnelling) and quantitative (shortening) changes of the cervix. These changes are recognised as cervical insufficiency. Intraamnial microbial colonisation occurs in as many as 80% patients with acute cervical insufficiency [12]. The aim of the present research is to "approach" the fetal compartment and to increase the accuracy of the diagnostics of intraamnial infection/inflammation.

The highest sensitivity in the experiment was achieved with the levels of anti-inflammatory cytokines (interleukins-6, -8 and -18) and matrixmetalloproteinases (MMP – 8, 9) in amniotic fluid; however, the examination is invasive and burdened with its own risk of complications. The elevation of interleukin-6 and MMP-8 in amniotic fluid is a more reliable diagnostic tool for already existing intraamnial infection/inflammation than the cultivation of amniotic fluid itself [13, 14].

The determination of MMP-8 in premature delivery has already been at disposal in the form of a bedside test and allows assessment of already existing intraamnial inflammation. The bedside test represents a quick semiquantitative test of the presence of the parameter observed. The test for the determination of MMP-8 is used in PROM [15].

A part of the research is focused on the study of serum maternal and umbilical parameters, which might be related to the development of premature delivery and FIRS. One of them is sRAGE. sRAGE is a soluble receptor for advanced glycation end products (AGE). This is a molecule from the immunoglobulin superfamily, whose ligands are, among others, the abovementioned oxidative stress products formed in the inflammation. RAGE occurs in a soluble (sRAGE) and transmebrane (RAGE) forms. RAGE is expressed on the surface of a number of cells (macrophages, monocytes, endothelial cells etc.). After the interaction of AGEs with the transmebrane receptor RAGE, the signal cascade including $p21^{ras}$ and MAP-kinase is initiated and followed by the activation of the nuclear factor kappaB. Its activation is followed by the stimulation of cytokine and growth factor gene transcription. The serum, soluble form of RAGE – sRAGE – has been studied in relation to the inflammation inhibition. High sRAGE concentrations might play a protective role in the development of inflammation [16, 17].

A significant field for research represents also the study of genetic predispositions for premature delivery; as mentioned above, an important part of the research is cytokine polymorphism, e.g. that of sRAGE or TLR. Some works emphasise the importance of gene interaction with the environment. Romero is dealing with the relationship of cytokine TNF-alpha (allele 2) polymorphism and occurrence of bacterial vaginosis with the development of premature delivery. The work gives evidence that women with TNF-alpha gene mutation and present bacterial

vaginosis are at 10times higher risk of premature delivery than those with bacterial vaginosis without the abovementioned mutation [2].

Uteroplacental ischaemia

The problem of the involvement of uteroplacental ischaemia in the ethiopathogenesis of premature delivery represents a relatively newly studied area. Again, the first work dealing with this problem was published by Professor Romero's team [18].

Concerning other studies, there is a rising trend in the number of evidence of the relationship between thrombophilias and premature delivery. In congenital (thrombophilic mutations) or acquired (anti-phospholipid syndrome) thrombophilic conditions, we assume an excessive coagulation activity with a potential effect on placental micro-circulation; the related endothelial dysfunction initiates a cascade of biochemical processes resulting in premature delivery. As mentioned above, PRR activation is assumed. The detailed mechanism has not been described yet. The most significant thrombophilias include Leiden Mutation, mutation of coagulation factor II – prothrombin, and anti-phospholipid syndrome. These coagulation disorders play a role in other pathological conditions in pregnancy, such as thromboembolism, intrauterine fetal death, intrauterine growth restriction (IUGR), placental abruption, severe pre-eclampsia and multiple spontaneous abortions (the role of thromboembolism to be found in literature). MTHFR mutation has not been considered significant until recently. Present works show that even this folic acid metabolism disorder might be, probably in combination with other factors, related to premature delivery. The metabolic transformation of folic acid results in the formation of an active vitamin – 4-THF-metafoline. One of the enzymes involved in its metabolism is methyltetrahydrofolate reductase (MTHFR). MTHFR gene polymorphism is connected with the reduction of their enzymatic activity, particularly in homozygote constitution, resulting in reduced formation of active folic acid metabolite, 4-THF. 4-THF represents a catalyst of the remethylation of methionine into homocysteine. Its lack (MTHFR mutation) causes insufficient homocysteine transformation, followed by its accumulation in the body. The homocysteine elevation is connected with endothelial impairment. Hyperhomocysteinaemia is a significant risk factor for arteriosclerosis. In MTHFR mutation carriers, it is assumed that the above mentioned mechanism might be related to placental micro-circulation impairment and to all other consequences (abortion, premature delivery, IUGR, sudden death syndrome). This area has now been studied intensively; however, there is no bigger study so far [19, 20, and 21]. There is evidence of the relationship between absolute (insufficient intake) or relative (MTHFR) lack of folic acid and congenital fetal disorders. The elevated concentration of "toxic" homocysteine in the body is considered as one of the main causes of the development of congenital disorders of the neural tube and heart [22].

The role of gestagens

The uterus belongs to the organs formed by smooth muscles; its physiology includes the same processes as those of other human organs of the same structure. However, some biochemical processes running in this organ have specific features. To understand the pathophysiological processes, it is necessary to remind the basic features of its physiology.

The principle of its function is contraction, resulting from the interaction between actin and myosin – proteins of muscle cells – myocytes. Particular myocytes are connected with gap and tight junctions, involved in co-ordination and synchronisation of the contractions in the whole uterus. The key process of the interaction between actin and myosin is myosin phosphorylation mediated by myosin kinase. The activation of myosin kinase requires the complex calmodulin and calcium ions. Calcium ion homeostasis plays a key role in the activity of myocytes. Intracellular calcium levels are regulated by two mechanisms: cell membrane influx and release from depots in myocytes (the sarcoplasmatic reticulum). Calcium ions enter the cells by at least two pathways: current-dependent involving cell membrane depolarisation and non-current-dependent involving the stimulation of the receptors with proper agonists or by the blockage with antagonists. The membrane receptors are formed by the action of ovarial and placental steroids. The action of progesterone results in the formation of type 2 – receptors = beta-adrenergic receptors. The action of estradiol causes the formation of type 1 receptors = alpha-adrenergic, muscarinergic, cholinergic, oxytocin and prostaglandin ones. The calcium influx into the cells is stimulated by type 1 – receptor agonists. The contraction is followed by the return of calcium ions into previous deposits against the concentration gradient by means of ATP-pump. The return of calcium is stimulated by type 2 – receptor agonists – beta-mimetics and magnesium. In addition, an important process is the regulation of myosin kinase by cAMP (cyclic adenosine monophosphate), which inhibits its function. The cAMP levels are elevated by the stimulation of type 1 – beta-adrenergic receptors, e.g. beta-mimetics. The transport of calcium ions into the cytoplasm and thus the activation and relaxation of the contractile system depends on the amount of the receptors and concentrations of proper agonists and antagonists.

The uterine contractility is changed during pregnancy and after the delivery onset. The initiation of delivery, connected with the expression of contraction-associated protein (CAP) genes, essential for the development of the uterine contractility, follows a relaxed pregnancy period with prevailing inhibition of the uterine contractility. The expression of CAP genes results in the production of an important protein – Connexin-43 – the main "building stone" of gap junctions and receptors. It is also part of the structure of ion channels. The regulation of the uterine activity in the relaxed pregnancy period – ensuring of the relaxation of uterine muscle tension – has still been studied at present. The most important

agents, discussed due to this regulation, are progesterone, relaxin, prostacycline (PGI_2), nitrogen (I) oxide and CRH (corticotrophin-releasing hormone). The action of these agents causes the elevation of intracellular cAMP, which both inhibits the release of calcium ions from intracellular deposits and inhibits myosin kinase, as mentioned above. However, normal uterine activity takes place; so called Braxton-Hix contractions occur mainly in the second part of pregnancy. Pregnant women might percept them as occasional "induration" or rare soft pain in the abdomen.

This preparation initiating phase is followed by myometrial stimulation with various agents – uterotonics – oxytocin, prostaglandins and probably also CRH – during the first and second phases of the delivery. After the delivery of fetus and placenta, the uterus is mainly stimulated with oxytocin.

Prostaglandins play a specific role. They are produced by enzyme prostaglandin synthase (PGHS) from arachidonic acid in amnial, chorial, decidual and myometrial cells. Recent research suggests that activity of this hormone is controlled by CRH, probably of the placental origin [23].

The amount and production of prostaglandins – the ratio of active agents and their metabolites – is controlled by enzyme prostaglandin hydrogenasis (PGDH) in chorions and placenta. Its activity is stimulated and inhibited with endogenous corticoids and progesterone, respectively.

Progesterone is the most significant hormone for pregnancy "maintenance". The previous paragraphs describe the role of progesterone produced physiologically in placenta. Both in vitro and in vivo evidence suggest that it inhibits the uterine contractility. The exact relationship between its levels and regulation of the number of receptors at the beginning of delivery has not been known yet.

First reports on the relationship between the additive administrations of gestagens in pregnant women to prevent premature delivery are in various studies from 1960s–1980s. These studies had probably improper design; the results were disputable and the conclusions were not transferred into practice in a wide range. The meta-analysis of the works from the year 1990 suggested that gestagens might have a favourable effect in the reduction of premature delivery risk [24]. However, the true progress in this area was not made earlier than in the works by Meis, da Fonseca and de Franco, suggesting the significant reduction of the incidence of premature delivery in patients treated with vaginal or oral gestagens, compared with placebo [25, 26, 27, 28].

The studies had a clinical character; none of them provided a reliable explanation of the mechanism of action of the gestagens administrated. At present, the hopes are targeted to the newest research of the relationship between the metabolism and action of gestagens and maturing procedures in the cervix. An animal study with mice suggests the effect of gestagens on the expression of some genes, particularly those of medroxyprogesterone acetate and progesterone [29].
The protein claudin (with 24 different types) belongs to the most important

transmembrane molecules of tight junctions. The tight junctions represent the structures, modulating intercellular communication. One animal study was concluded that claudin gene expression is reduced in cervical epithelial cells during both premature and termed delivery [30]. The study of Timmons et al. brings evidence of claudin gene upregulation and thus of its increase after the administration of gestagens. Although this is an animal model, it can be concluded that claudins play a key role in cervical maturing processes. The work supports the assumption that the administration of gestagens is a potential preventive precaution in the risk of premature delivery [31].

Fetus as an allograft

The presence of abnormal immunological processes in the ethiopathogenesis of premature delivery has already been studied for many years. There are various kinds of evidence (at molecular as well as at genetic level) supporting the assumption that premature delivery and/or multiple abortions can occur due to fetus as an allograft. However, classical transplant theories do not provide satisfactory explanation. The essential mechanism of maternal tolerance of the fetus is a "subtle" balance between downregulation and upregulation of the main MHC antigens (MHC = Major Histocompatibility Complex) [32]. While the genes for class I HLA-A and -B antigens are downregulated by trophoblasts (genes are not expressed), the genes for class HLA-G antigens, protecting the fetus against maternal immune response, are expressed during pregnancy [33]. Non-adequate identification of fetal antigens by the mother might result in failed maintenance of the pregnancy. However, exact continuity has not been known yet. A number of other works deal with connecting links of the immune response at various levels. For example, animal studies showed reduced expression of galectin-1 (an immunoregulation protein) in multiple fetal losses.

A number of autoimmune diseases are – when uncontrolled – also connected with a higher risk of premature delivery. The most common ones include ulcerous colitis, lupus erythematodes and thyroid gland immunopathologies (TRAK or TPO antibodies) etc. [34, 35].

Allergy

The participation of allergy in the pathophysiology of premature delivery has been demonstrated in various works. For example, it is known that the uterus is a rich source of mastocytes – one of the "executive" cells of allergic reaction; pharmacological degranulation of mastocytes results in the induction of uterine activity, particularly prostaglandin release [36]. Another work describes the occurrence of eosinophillic granulocytes in amniotic fluid obtained from women with premature delivery, compared with the control group. The presence of eosinophiles supports the presence of abnormal immune/allergic response as one of the ways to premature delivery; however, the antigen triggering this reaction

has not been found yet. Similarly, a possible role of antihistamine agents in the prevention of this type of premature delivery remains an open question [37].

Excessive uterine expansion
Congenital uterine disorders, polyhydramnion and multiple pregnancies are connected with the risk of premature delivery. The intrauterine tension remains relatively constant during the whole pregnancy period, despite the uterine growth. This phenomenon is mainly explained by the action of progesterone and endogenous myometrial "relaxing agents", particularly nitrogen (I) oxide (NO). However, the excessive uterine expansion caused by the above mentioned situations results in the increase of myometrial contractility, prostaglandin release and expression of gap junctions proteins, particularly Connexin-43. Similarly, excessive amniochorial expansion results in mechanic damage of chorions, potentially resulting in PROM. At present, this way to premature delivery is most difficult to control.

Cervical incompetence
Insufficient cervical closing ability occurs in various forms in pregnant women. This diagnosis is connected with abortions in the second trimester and premature delivery and, with the same probability, with headlong termed delivery. The congenital causes include rare cervical hypoplasia and diethylstilbestrol (DES) exposure of the mother. The acquired insufficiency has an origin particularly in previous cervical surgery. The examples are cervical conization and multiple endocervical dilatations connected with pregnancy interruption (abortions). Other potential mechanism is represented by intrauterine infection (MIAC). As mentioned above, a high rate of women with MIAC have also cervical incompetence.

These findings also suggest a change in the view of the use of endocervical cerclage. The aim of Romero's meta-analysis was to answer the question of the role of cerclage in secondary prevention of premature delivery. The results did not bring reliable evidence of the benefit of routine planned endocervical cerclage until 23rd week of pregnancy in women at high risk of premature delivery due to the history (the history of premature delivery or abortions). It is recommended to indicate women for this surgery selectively, indicating those with dynamic changes in ultrasound cervicometry parameters. However, there are doubts in these situations, too, regarding the high incidence of MIAC. In the future prospect, it can be assumed that the decision about the indication for this surgery will depend on the presence of MIAC. At present, there are no reliable non-invasive methods for elimination of intrauterine microbial colonisation/infection. Romero suggests using the assessment of interleukin-8 levels in cervical fluid combined with ultrasound cervicometry. With low interleukin and thus low risk of intrauterine infection, the women might be offered the cerclage with cervical conization. Other non-invasive alternative is the abovementioned vaginal administration of gestagens [38].

Conclusion

In the past recent years, perinatological care introduced significant changes in the care of both pregnant women and new-borns in diagnostic and therapeutic fields. New knowledge of pathophysiological mechanisms enables us to include the new procedures into prenatal and peripartal care. Birth canal infections remain the most significant trigger mechanism of premature delivery; the related biochemical changes significantly affect perinatal morbidity and mortality. Other potential mechanisms, particularly disorders of hormone metabolism and action and uteroplacental ischemia have been intensively studied. Together with infections, they can be controlled most easily. The particular pathogenic process of premature delivery is inflammation. This process affects both mother and fetus. Fetal inflammatory response (FIRS) – possible even without maternal response – is related to higher perinatal morbidity. FIRS is characterised by defined laboratory, histological and clinical criteria. There is no effective primary prevention of premature delivery at present. The sensitivity and specificity of the laboratory markers having been used so far is low. Thus, the research is focused on the finding of new markers to ensure early identification of a group of pregnant women at high risk of premature delivery and fetal inflammation. Screening of women at high risk by new laboratory and ultrasound tests represent the most important steps in secondary and tertiary prevention of premature delivery. The present perinatological research invades the field of molecular biology. Intensive research of other potential trigger mechanisms of premature delivery has been carried out, including various gene types, whose abnormal expression is potentially connected with the process of premature delivery.

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