Review Article

# Gestational Inflammation: Its Foetal Control and the Proper Therapeutic Approach

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**Inflammation triggers coagulation. Gestational inflammation, with its vascular implications, is the cause of major obstetric complications, from sporadic and recurrent miscarriage to foetal growth restriction, premature delivery with all its nefarious perinatal sequels, and the most dramatic scenarios of foetal and maternal death: it is neither adequately diagnosed nor promptly and exhaustively counteracted by current obstetrics.**

**Controlling gestational inflammation in a normal pregnancy is primarily a foetus's job. The vast majority of aneuploid foetuses are unable to control normal gestational inflammation and are therefore spontaneously aborted. Euploid foetuses may be unable to control normal gestational inflammation, and in this case, they are aborted or suffer from the inflammatory complications of advanced pregnancy.**

**The present article explains the reason for choosing betamethasone for the prevention and therapy of gestational inflammation. At the right dose and for the right time, its administration before and during pregnancy does not cause negative effects on the foetus or the mother. Free prescription of betamethasone for the protection of gestation is suitable even for patients with no history of previous pregnancy loss. In addition to women undergoing in vitro fertilization and embryo transfer, even women in advanced age, at their first apparently normal spontaneous pregnancy, are candidates for betamethasone protection, simply because many of them will not be given a second chance to become mothers.**

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## Premise

It is worth considering in advance that pregnancy is made possible thanks to an invasion of the maternal endometrium by the trophoblast surrounding the blastocyst. Such invasion generates a cellular reaction[1], the nature of which is primarily of an inflammatory type. It belongs to the cellular branch of the immune response, leaving the humoral one perfectly operative.

A favorable outcome is only possible if the trophoblast is able to turn off the inflammatory endometrial reaction it itself has caused. This means that the physiological pregnancy is largely under foetal control. Foetuses (i.e., trophoblast) unable to properly modulate the local maternal reaction sign their own death warrant.

## The physiological role of the 'inflammatory mediators'

The normal evolution of pregnancy is the result of deep vascular changes in the maternal organism under the control of foetal mediators. According to their prevalent action, a taxonomic classification can divide these mediators into inflammatory and anti-inflammatory. However, they are known to play either role, depending on the cell type and the circumstances of their involvement, in response to the activation of the actors of the opposite function. Indeed, the so-called 'inflammatory' ones primarily regulate physiological functions, such as ovulation, menstruation, labor, and many others. In search of scientific evidence of the physiologic role of such mediators, we found that receptor ligands for the inflammatory peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP) are present in amniotic fluid. Their concentration increases with labor, while fMLP receptors are expressed in amnion tissue[2][3][4][5]. So, what are these inflammatory mediators for? Can normal labor be considered an inflammatory process? Not entirely. Certainly, it often presents with pain, heat, and functional impairment, like it may happen during menstruation: and to some extent, labor can be considered a delayed menstruation, as Gustavii pointed out a long time ago[6].

Is fMLP involved in the control of menstruation as well? After all, understanding the physiological role of these mediators is not a simple matter of measurement. fMLP is only one among many chemotactic factors able to modulate cytokines, including IL-1 alpha and IL-1 beta gene expression and IL-6 secretion, which in turn are known to modulate inflammation[7].

Therefore, it should be interesting to explore the behavior of the peptide in the genesis of premature labor and foetal systemic inflammation, a syndrome preceded by an increase of IL6 concentration in the foetal

compartment. However, even if this were the case, it would be a drop in the ocean! A large network of gestational cytokines, chemokines, peptides, and prostanoids have been investigated in both experimental and clinical conditions, with sometimes controversial results. Nevertheless, over the course of half a century, the inflammatory nature of the major complications of pregnancy has been widely recognized, at least on a physiopathological level, if not on a clinical one.

In this article, based on the most relevant scientific literature, as well as on fifty years of experience as a researcher and clinician, I will discuss the concept of gestational inflammation in its broadest sense, from the natural maternal immune response to its physiological control by the foetus, up to its harmful pathological deviations. Furthermore, since the imbalance of these mediators precedes inflammation, triggers it, and paves the way for infection, I will also discuss the therapeutic approach I adopted for the prevention and treatment of their deviations.

## A look at the nature and mechanism of gestational inflammation

Between the two endpoints of conception and delivery, the process of placentation takes place, during which the maternal and foetal immune systems interact with each other. This occurs through the release of specific mediators of opposite function, the maternal ones being more addressed towards inflammation, whereas the foetal ones act against it. The earliest stage of the foetal-maternal competition occurs at the beginning of blastocyst implantation in the uterine mucosa. Mainstream clinicians are accustomed to considering such an early stage separately from advanced pregnancy, when the foetus is now capable of extrauterine life. Nevertheless, both from a biological and clinical point of view, the functional evolution of the trophoblast must be considered a unitary and continuous process until delivery. Indeed, the same inflammatory deviation of its function that triggers miscarriage can later lead to premature birth, chorioamnionitis, nefarious perinatal complications, up to the loss of both the foetus and the mother.

During the three-day travel from the tube to the uterine cavity, the product of conception does not need to come into contact with the maternal blood, since everything it needs is already in its baggage. Later, when the trophoblast cells reach the mucosa, they differentiate into two types, respectively called extra-villous and villous. The first type is responsible for structural and functional changes in the maternal vessels[5]. It penetrates the uterine arterioles and disrupts their wall, causing the leakage of blood in which the chorionic villi are immersed to drain increasing quantities of nutrients and oxygen.

As in any other vascular district of the body, this generates an inflammatory reaction, aimed at reducing blood leakage by means of vasoconstriction and coagulation. The activation of such a natural response would appear to hinder the ultimate goal of a normal birth, but in physiological pregnancy, this is not the case. In fact, the foetal immune system takes over the maternal one to preserve optimal placental perfusion until childbirth. In its competition with the maternal immune system, foetal self-defense is essentially accomplished by counteracting vasoconstriction and blood coagulation. For this purpose, extravillous trophoblast cells progressively eliminate the smooth muscle from the wall of the spiral artery up to its myometrial tract. The consequent vasodilatory effect produces a continuous, low-resistance blood flow. At the same time, coagulation is prevented by replacing the endothelial lining of the vascular intima with trophoblastic cells. In this way, the local maternal platelet activation does not take place, and thrombosis is not triggered.

These structural changes of the uterine arterioles were clearly described by Harold Fox in his classic text entitled 'Pathology of the Placenta' *(ISBN 13:9780702021961)*.

At the same time, the trophoblastic cells directly produce functional mediators and stimulate the release of many others by maternal platelets, as well as by immune and other cells. Therefore, it is right to state that pregnancy is directly under the control of the foetus itself: *foetus faber fortunae sua*e!

On the other hand, contrary to some unproven opinions, maternal immunity against infectious diseases remains fully operative throughout pregnancy. The idea of an assumptive "fragility" of the pregnant woman is completely devoid of any scientific basis. It derives solely from the hypothesis that the foetus should be 'rejected' because the mother's immune system does not recognize its paternal genetic component. According to this absurd opinion, maternal tolerance would be accomplished through a reduction of humoral immunity, thus leading to an increased risk of infection. A corollary of this unproven risk should be the need to resort to vaccines, whose number is continuously increasing, and whose efficacy and safety have never been reliably investigated. Instead, contrary to the opinion of an increased risk of serious infections accredited by the World Health Organization, the admission rate to Intensive Care Units is significantly lower for pregnant women. Moreover, no increased need for ventilatory support, nor a greater likelihood of severe outcomes and maternal death compared to either the general population or non-pregnant women of reproductive age is reported. Only a higher risk of hospitalization was registered, rightly ascribed to better care for motherhood[8].

Surprisingly, however, the above data have been interpreted in the opposite meaning by others[9]. To give greater credence to an increased risk of maternal death, these authors go back to the 1918 flu pandemic,

an era when the therapeutic efficacy of antibiotics, anticoagulants, and anti-inflammatory drugs was unknown!

Unfortunately, however, vaccination, by definition, entails inflammation, thus strengthening the normal maternal cellular immune reaction[10], with a consequent increased risk of foetal demise. It is the author's opinion that, given the current uncertainties, it may be best to exercise caution regarding vaccinations during pregnancy until more research is available.

## Foetal-maternal regulation of immune and vascular function

Many of the pregnancy-related mediators of cellular functions are secreted by two subpopulations of lymphocytes (Th1 and Th2) derived from the functionally undiscriminated type (Th0). Th1-type produces mainly interleukins (IL-1, IL-2, IL-12, IL-15, IL-18), interferon gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), known to mediate cellular immunity. Th2 produces IL-4, IL-5, IL-6, IL-10, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which control the humoral response[11][12].

Moreover, Th2 cytokines prevent Th1 cytokine secretion[13].

The pro-inflammatory/anti-inflammatory cytokine ratio is enhanced in preeclampsia. It is probably associated with an excessive production of inflammatory agents, among which IL-12[14]. Placental ischemia, consequent to poor spiral artery remodeling[15], enhanced Th1/Th2 ratio[16] and pro-angiogenic/anti-angiogenic factor imbalance[17] may promote inflammatory changes through the release of Th-1 cytokines and ROS with consequent endothelial dysfunction, leading to the release of humoral factors responsible for the clinical symptoms of preeclampsia[18].

As for the gestational evolution of cytokine balance, based on circulating levels of interferon-gamma (IFN-gamma) and interleukin-6 (IL-6) as Th1 and Th2 biomarkers respectively, the variation of their ratio was investigated in the maternal blood of 35 women from 10 to 40 weeks of pregnancy. It is important to note that IFN-gamma levels decrease with gestational age, whereas those of IL-6 increase. The IFN-gamma/IL-6 ratio switches around the 19th week of pregnancy. Therefore, the authors suggest that the Th1 bias may be prevailing at the beginning of pregnancy, balanced in the middle, and supplanted by the Th2 bias at the end[19].

These results should be taken into account when the cytokines are tested for statistical or diagnostic purposes.

Many other factors are involved in the differentiation and specialization of trophoblastic cells from implantation to delivery. During early pregnancy, low oxygen is reported to prevent trophoblast differentiation towards an invasive phenotype. Low oxygen effects are mediated by hypoxia-inducible factor-1 (HIF-1alpha subunit), whose expression parallels that of transforming growth factor-beta3 (TGFbeta3), an inhibitor of early trophoblast differentiation[20] [21].

Abnormalities in TGF-β3 expression are associated with preeclampsia, and it has been demonstrated that down-regulation of this growth factor restores the invasive capability of preeclamptic trophoblast cells[22].

A crucial role for optimal fetal oxygenation and growth is exerted by prostacyclin ($PGI_{2)}$, a prostanoid with vasodilatory and anticoagulant function. It is produced by both the mother and the fetus for the entire duration of pregnancy. Placental cells in culture synthesize and release $PGI_2$. Its production appears to increase with gestational age. Moreover, preterm and term placental $PGI_2$ production is significantly greater than that of the first trimester. Notably, synthesis of $PGI_2$ is inhibited by the cyclo-oxygenase inhibitors indomethacin and aspirin, a potentially dangerous effect, considering the wide clinical use of these drugs[23].

Indeed, as patients with recurrent miscarriage have significantly increased platelet aggregation in response to arachidonic acid, the use of aspirin in the management of this condition is suggested[24].

The action of prostacyclin is normally counterbalanced by thromboxane (TxA2), a prostanoid responsible for vasoconstriction and thrombosis. TxA2 is released by various cells, among which are endothelial cells, macrophages, and neutrophils. The enhancement of thromboxane release is the natural consequence of inflammation. The maternal inflammatory response is not limited to platelet activation but widely affects other tissues, among which is the endothelial lining of uterine arteries. Women whose pregnancies end in abortion have a higher output of 2,3-dinor-$TxB_2$ (a thromboxane metabolite) and lower excretion of 2,3-dinor-6-keto-$PGF_{1\alpha}$ (a prostacyclin metabolite), with a lower ratio of prostacyclin to thromboxane, indicating a thromboxane dominance and prostacyclin deficiency compared with women whose pregnancies proceed to term[25].

These data underline the importance of a balanced prostacyclin/thromboxane ratio in pregnancy.

In contrast with the generally accepted idea that increased platelet aggregation is a cause of severe complications, among which are increased miscarriage rates, pre-eclampsia, and pregnancy loss, reduced platelet aggregation in response to adenosine diphosphate and thrombin receptor-activating peptide was

reported in patients with a history of recurrent miscarriage in comparison with those of the same group who subsequently have successful pregnancies. Women with subsequent miscarriages also have a trend towards reduced platelet aggregation in response to epinephrine[26].

However, in vivo platelet dysfunction is a complex process that cannot be simply reduced to more or less experimental in vitro aggregation.

Further questions regarding platelet dysfunction concern the complexity of the maternal inflammatory response and the adequacy of the adopted therapy.

## Potential causes of enhanced gestational inflammation

Once the prevalent inflammatory nature of major obstetric complications is understood, its prevailing causes must be carefully considered. In this regard, the current attitude is to look for maternal infectious, immune, or genetic causes.

As for the first, the stubborn search for potential pathogens is no longer necessary, since the fetus's inability to turn off the maternal immune reaction - unleashed by itself - is sufficient to explain even the most serious consequences. After all, for instance, free mother-to-child transmission of viruses and their specific antibodies has been reported in normal pregnancy[27][28]. Moreover, HPV infection is rare in chorionic villi and is not related to abortion[29]. As for Ureaplasma parvum and urealiticum, Mycoplasma genitalium and hominis, and Chlamydia trachomatis, their DNA sequences are found with similar frequency in peripheral blood mononuclear cells of spontaneous and voluntary aborters[30].

On the other hand, it is logical to believe that the mere presence of viruses and bacteria is not a sufficient cause of disease, because it is known that healthy carriers exist. The independence of the onset of inflammation from pathogens is indirectly demonstrated in the above-mentioned ‘syndrome of the systemic foetal inflammatory response’ (Romero – Gomez Syndrome). In this condition, high levels of foetal IL6 are found in the foetal compartment before the onset of premature birth symptoms, suggesting the existence of a foetal cytokine imbalance preceding the eventual infection. Nevertheless, the widely held belief that inflammation is triggered by infection is claimed on the basis of the curative effect of antibiotics. However, we have shown that, in the absence of infection, ampicillin is able to directly inhibit amniotic Prostaglandin E[31] and IL6 release[32].

Furthermore, we showed that Ceftriaxone and Gentamicin exert the same action, although to a lesser extent. On the contrary, Tetracycline and Erythromycin do not influence the prostanoid output. The

inhibitory (i.e., anti-inflammatory) effect of Ampicillin is potentiated in an additive manner by Ceftriaxone, reduced by Gentamicin, and eliminated by Tetracycline and Erythromycin[33].

These data should open a serious debate on the current interpretation of healing from antibiotics: is it the result of their direct anti-inflammatory action rather than the anti-bacterial one? Or: to what extent is it the result of both?

Autoimmune syndromes and diseases of the maternal connective tissue are a well-known cause of recurrent miscarriage. Nevertheless, successful pregnancy is possible, although at the cost of an increased risk of complications[34].

## The foetal-maternal genetic control of gestational inflammation

A large number of maternal and foetal cells produce immune mediators, but a clear assessment of their role in the genesis of different gestational situations is not easy. While the involvement of the maternal immune system is beyond question, the foetal control over it has not been adequately understood. There is much evidence to suggest that the foetus itself governs the balance of gestational immunity. For example, the Syndrome of the Foetal Systemic Inflammatory Response (hence premature delivery, chorioamnionitis, and their related perinatal complications) is characterized by a high level of foetal interleukin-6, whose increase precedes the onset of symptoms[35], This indicates that the cytokine imbalance is not a result of infection. Accordingly, it has also been postulated that successful pregnancy induces an immune bias towards Th2 immunity[36], and a Th2 cytokine imbalance is believed to cause intrauterine growth restriction and foetal death[14].

Increased or unbalanced release of pro-inflammatory mediators appears to be triggered by the foetus itself. Indeed, maternal peripheral mononuclear cells stimulated with trophoblast antigens from growth-restricted foetuses produce higher levels of pro-inflammatory cytokines compared with normally grown foetuses[37].

Growth restriction, therefore, can be considered a consequence of low utero-placental perfusion caused by vasoconstriction and thrombosis of uterine spiral arterioles: i.e., by the normal maternal inflammatory response, which growth-restricted foetuses lack the ability to switch off. Thus, it is believed that 'unexplained' pregnancy failure is a result of the shift towards Th1-type immunity[38].

Once the foetus' inability to properly control the maternal inflammatory reaction has been recognized, we must find out where it originates from. To answer this question, we investigated the mediators of

inflammation, coagulation, and vascular function released by aneuploid foetuses, in order to verify whether an increased or unbalanced output could justify their high abortion rate.

We found a raised amniotic fluid level of endothelin, a potent vasoconstrictor, in the presence of aneuploidy compared to normal pregnancies[39]. Moreover, we registered abnormalities of the plasminogen activation system, possibly leading to impaired utero-placental perfusion, thus to abortion, foetal growth restriction, and death[40].

Anomalous amniotic and maternal plasma levels of growth factors and proinflammatory cytokines in the embryo are correlated with chromosomal alterations[41].

In particular, down-regulation of adhesion molecules such as integrin $\alpha$, and upregulation of MMP-9 are reported in trisomy 21, with possible impairment in trophoblast function. These alterations may be responsible for the increase in cytotrophoblast apoptosis[42]. Moreover, we registered an increase of interleukin-6 ($P = 0.034$) and a decrease of interleukin-8 ($P <$ or $=0.0001$) in the amniotic fluid of aneuploid foetuses[43].

Finally, we investigated the receptors of adenosine, a proangiogenic nucleoside, in 71 first-trimester chorionic villi samples and cultured mesenchymal cells from euploid and trisomy 21 pregnancies. Overall, the adenosine transduction cascade appears to be disturbed in Trisomy 21 through reduced expression of $A_{2B}$ and $A_1A$ receptors[44].

All these anomalies of mediators of inflammation, coagulation, and vascular function may cause foetal growth restriction, malformation, and death, thus leading to the high rate of aneuploid pregnancy loss.

If the above-reported evidence speaks in favor of a foetal genetic cause for the lack of a proper control of immune and vascular function in the presence of aneuploidy, what can be said for euploidy?

Progress in genetic polymorphisms research answers this question. Genetic polymorphism means that the structure or nucleotide arrangement of the same gene may vary between individuals. The variations are compatible with life and may affect numerous genes, including those associated with angiogenesis, thrombogenesis, and immune response[45].

Maternal common inflammatory polymorphisms entail an increased risk of recurrent miscarriage[46] and preterm birth[47][48].

Consistent with the increased risk of preterm birth that inflammatory polymorphisms entail, a decreased risk is reported for the anti-inflammatory ones[48].

Furthermore, maternal polymorphisms of the immunoregulatory genes IL10, MBL2, TNFRSF6, and TGFB1 influence the susceptibility to chorioamnionitis and other unfavorable pregnancy outcomes [49].

While maternal polymorphisms are widely studied, the same cannot be said for the foetal ones. This reflects the current and often erroneous opinion that adverse pregnancy events are attributable to maternal pathological conditions. On the contrary, since both the maternal and foetal immune systems modulate the utero-placental function, foetal genetic polymorphisms should have the same importance as maternal ones in determining the fate of the pregnancy.

## Clinical implications of the foetal-maternal immune system mutual modulation

The evidence reported above is only a small part of the large amount of data in the scientific literature on cytokine modulation in pregnancy. As mentioned in the introduction, the maternal inflammatory response is not in itself a pathological event, as far as the foetus is able to counteract it. It turns into pathology only when the spatial-temporal production of inflammatory cytokines is uncontrolled, and the Th1 foetal-maternal immune response takes over the Th2 one[50].

So, slowly, the idea of counteracting inflammation has made its way into the minds of obstetricians. It must be said, however, that the maternal immune reaction to trophoblast invasion is not currently seen as a possible cause of miscarriage. According to mainstream obstetrics, the most accredited causes are maternal diseases, including connective tissue chronic inflammation, thrombophilia, and infection. Indeed, an increased risk of miscarriage and other complications is registered in the presence of such pathological conditions. Nevertheless, physiological pregnancy, albeit less frequently, can occur in these patients, thus confirming the opinion that a better genetic polymorphism of the new foetus may better control the maternal immune system.

Ignoring the possible role of gestational inflammation in the genesis of obstetrical complications, current obstetrics does not effectively counteract it. Obstetricians remain basically anchored to the weak measures dating back to the first half of the last century: robust doses of progesterone, weak doses of aspirin, and in some cases, ineffective glucocorticoids.

Progestagens are prescribed routinely for the protection of early and late pregnancy. Initially, the reason for their use was the maintenance of electrical and mechanical quiescence of the myometrium[51].

As for their utility, progesterone's action on the endometrium for implantation is known and beyond question. Therefore, its use can be justified in the preparation of the uterine decidua in the imminence of the embryo transfer and during the early stages of its evolution. More recently, a possible anti-inflammatory action of the hormone against miscarriage has also been reported[52]. It is worthwhile to recall that around the end of the last century, a combination of hydroxiprogesterone caproate plus estradiol valerate, named Gravibinan, was also approved for the cure of threatened miscarriage[53], for osteopenia due to hypogonadism,[54], for stimulation of breast enlargement[55], and also for contraception[56]. Only in 2007 was Gravibinan withdrawn from the Italian market. Fortunately, however, according to FIGO Good Practice Recommendations[57], evidence on the use of progesterone for the prevention of recurrent miscarriage demonstrates no significant difference in live birth rates compared to placebo. Similarly, a recent meta-analysis on seven randomised trials involving 5,682 women concluded that progestogens probably make little or no difference to the live birth rate for women with threatened or recurrent miscarriage[58]. Furthermore, the method of pessary administration of 400 mg vaginal progesterone for threatened miscarriage does not increase live birth rates[59]. Other authors, however, seem to interpret the recommendations of the guidelines in a more reassuring way[60]. As for the prevention of premature birth, its use is not recommended by the American College of Obstetrics and Gynecology[61].

The pathogenic link between inflammation and thrombosis, and their role in obstetric complications, guided some clinical preventive strategies. Based on the known deficiency in prostacyclin and overproduction of thromboxane A2 in women with a history of recurrent miscarriage, 50 mg of aspirin was daily given throughout the entire duration of pregnancy to 66 patients. The therapy was able to decrease thromboxane A2 production without influence on prostacyclin concentration, but no improvement in the live birth rate was observed[62]. Moreover, a meta-analysis evaluated the efficacy of aspirin and heparin in women with a history of at least two unexplained miscarriages with or without inherited thrombophilia. Nine studies, including data from 1,228 women, were reviewed. No beneficial effect of these anticoagulants in studies at low risk of bias was found[63].

As for corticosteroids, as far as I know, the first report of their obstetric use dates back to 1953, when they were given in cases of hyperemesis gravidarum due to a state of relative adrenal insufficiency. 29 women were treated with doses of cortisone varying from 25 to 75 mg/day for 5 to 64 days. 23 patients had a complete remission of the symptoms within 36 hours[64]. Today, corticosteroid prescription is

widespread during mid- and late pregnancy, while little is known about its potential significance in early pregnancy. Stimulation of hCG secretion, suppression of uterine natural killer cells, and promotion of trophoblast growth/invasion have been reported among the positive effects, while the negatives could be inhibition of cytokine-prostaglandin signalling, restriction of trophoblast invasion following up-regulation of plasminogen activation inhibitor-1, induction of apoptosis, and inhibition of embryonic and placental growth[65]. However, a large part of these adverse effects derives from laboratory experiments not confirmed by clinical studies. Furthermore, the results of experimental studies on animals cannot be transferred from one species to another, because each species reacts to glucocorticoids in its own way[64].

Curiously, the main role of cortisone, that is the anti-inflammatory one, does not seem to be the one that implements its clinical use in obstetrics!

## Personal experience with glucocorticoid therapy in pregnancy: the Irpinia Resolution

The guidelines are produced with the intent of providing quickly accessible information on the clinical trends that appear most promising. I suggest, however, that there is a risk that their rigid application may, in some cases, lead to inadequate care and potentially dangerous outcomes for the individual patient. As far as I'm concerned, at the beginning of my residency, I followed the therapeutic indications of my Professors. For prevention and therapy of threatened miscarriage, these were progesterone combined with smooth muscle relaxants. The same therapy was commonly prescribed even in conditions without specific risk, such as uterine retroversion or advanced maternal age, thus increasing the misunderstanding of both the real cause of complications and the real effect of therapy. However, I soon realized that for recurrent miscarriage, the therapy was always the same, despite its previous failures. At that time, the inflammatory nature of obstetric complications had not yet been extensively investigated. Nevertheless, the ineffectiveness of progesterone was so obvious that it could not be denied. With the introduction of ultrasonic diagnosis, after my attendance at the course held by Professor Alfred Kratockvil at the University of Vienna in 1977, I was in charge of echography in my Institute: the Ob & Gyn Unit of the University of Ferrara, Italy. This gave me the opportunity to monitor all the patients treated in my Department. So, I could realize that quite often, progesterone, blindly prescribed even after the foetal heartbeat had already disappeared, did transform a threatened miscarriage into a missed miscarriage. Since the alleged luteal insufficiency as a cause of miscarriage had never been proven in a reliable way, I

decided to stop prescribing progesterone for the protection of pregnancy. My gradual transition to cortisone took place slowly, through the complex path of scientific research and clinical experience. The first patient I treated successfully with Bentelan 0.5 mg was followed by hundreds of other women with a history of recurrent pregnancy loss. I clinically assessed over one hundred and fifty of these patients, and the results of my studies became the subject of peer-reviewed scientific publications.

On the one hand, with my coworkers, I deepened my knowledge on some effects of betamethasone on gestational tissues. My coworkers and I demonstrated, for instance, that the hormone induces the release of lecithin from the foetal membranes. This action suggests that the beneficial effect naively called 'maturation of the premature lung' is nothing but the result of foetal inhalation of the amniotic lecithin supplement released by the chorioamniotic membranes thanks to the power of 24 mg injected, coupled with the subsequent suppression of the foetal lung inflammation[66][67].

After all, how could a lung 'mature' within 24 hours if it has not done so in the preceding weeks or months?

In addition, we found that betamethasone, progesterone, and mifepristone, despite the opposite clinical action of the first two compared to the third, exert a similar in vitro effect on trophoblast connexin expression. This confirms that the in vitro biological effects may have nothing to do with the in vivo clinical ones[68].

On the other hand, while reviewing the biological and clinical aspects of glucocorticoids in pregnancy[69][70], we studied the effect on foetal growth of a low dose of betamethasone administered throughout pregnancy[71]. Contrary to the unfounded opinion that glucocorticoid assumption is a cause of foetal growth restriction, we found that out of a total of 160 treated patients with a history of recurrent miscarriage, 44 gave birth to foetuses weighing above the fiftieth percentile. While speaking against a betamethasone-induced impairment on foetal growth, our findings indicate that in the strenuous foetal-maternal competition, the hormone, although effective in ensuring survival, in some cases may not be able to guarantee the best foetal weight: in other words, without its help, those foetuses would not even have been born.

Moreover, since a higher concentration of Natural Killer (NK) cells is reported among the signs of inflammation, we computed their maternal peripheral blood number in cases with foetal growth restriction. Our finding was a higher NK percentage and absolute value in these patients[72].

Once the pathogenic mechanism of gestational complications and the efficacy of cortisone use had become clear to me, there were still many questions to be answered, among which were the eventual need for other drugs, and the right dose and duration of the therapy. Indeed, faced with the substantial inefficacy of progesterone, an increasing number of drugs were prescribed by others, including aspirin, thyroid hormone, heparin, and more recently vitamin D, prednisolone, and various 'supplements' (not to mention some modulators of the maternal immune system suggested by rheumatologists). The number of previous failures among my patients ranged from a minimum of two to a maximum of ten. They usually came to my attention after an incredible number of laboratory tests, hysteroscopies, excisions of uterine fibroids, cysts, and foci of endometriosis, and prolonged copious intake of drugs. How could I ever understand, ex juvantibus, the value of betamethasone if I combined it with other medicaments? My choice was therefore to limit the therapy to cortisone alone: if the cause is really inflammation, there is no better anti-inflammatory – I thought! Betamethasone 0.5 mg throughout pregnancy was generally effective in my experience. Nevertheless, there are cases where the dose must be modulated according to the clinical evolution (bleeding, pain, foetal growth, cervical length, blood test, maternal symptoms). Some patients living in distant regions received advice from other gynecologists to increase the dose and replace betamethasone with prednisolone: bad advice, which comes from ignorance and inexperience. Indeed, it is currently said that prednisolone would be preferable because it does not reach the foetus and therefore cannot have negative effects on it. Wrong! Prednisolone, like all steroids, crosses the phospholipid layer of the cell membrane, entering the trophoblast. The reason why it is little or not at all effective lies in its poor anti-inflammatory activity, which is further reduced by 11-beta-hydroxy-steroid-dehydrogenase abundantly present in the chorion-decidual tissue. As it is known, on the contrary, the anti-inflammatory activity of betamethasone is much greater. Furthermore, like other fluorinated glucocorticoids, it is insensitive to the enzymatic action of dehydrogenase and therefore exerts its action undisturbed exactly where it is required, that is at the level of the trophoblast (indeed, the foetus is the first target of the therapy, not the mother!). The anti-inflammatory clinical weakness of prednisolone is clearly evident in a patient with a history of recurrent miscarriage. She had been treated with low-dose aspirin daily for three years without any result. Due to an excessive number of uterine NK (30%), after the fourteenth failure, she was treated with preconceptual prednisolone 5 mg, without a favourable outcome. Following the nineteen abortions, she received preconceptual prednisolone 20 mg/day for 6 months, thus becoming able to carry the twentieth pregnancy up to the thirty-second week, when she gave birth by caesarean section to a growth-restricted foetus[73]. This interesting case was considered of 'untested

significance' by the Authors. However, one significant remark can be made: when a decision is made to treat gestational inflammation, the right dose of the effective drug should be given for the right time.

So, the next question can be: what is meant by 'right time'?

In my experience, the answer is that gestational inflammation must be controlled for the entire duration of pregnancy. Indeed, it can arise slowly, be of mild degree and chronic evolution, or it can explode acutely, with a stormy course, even in advanced pregnancy and during labor. I myself made attempts to reduce or discontinue betamethasone at various stages of pregnancy, quite often with negative results. But then I asked myself: why stop the drug if it never produced foetal-maternal negative effects? So, in the last decades, my advice to patients has always been to continue the therapy until the moment of having the baby in their arms.

## Concluding remarks

There would still be much to say, but it is time to come to the conclusion.

Based on more than forty years of experience with the use of betamethasone in the prevention of pregnancy loss, I must emphasize the following points:

- Uncontrolled gestational inflammation, with its vascular implications, is the main cause of the major obstetric complications, from euploid and aneuploid sporadic and recurrent miscarriage to foetal growth restriction, premature delivery with all its nefarious perinatal sequels, up to the most dramatic scenarios of foetal and maternal death.
- Gestational inflammation has not been correctly interpreted by current obstetrics and therefore is neither adequately diagnosed nor promptly and exhaustively counteracted.
- Controlling normal gestational inflammation is primarily the foetus's job.
- The vast majority of aneuploid foetuses are unable to control normal gestational inflammation and are therefore spontaneously aborted.
- Euploid foetuses may be unable to control normal gestational inflammation, and in this case, they are aborted or suffer from the inflammatory complications of advanced pregnancy.
- Betamethasone is the drug of choice for prevention and therapy of gestational inflammation. At the right dose and for the right time, its administration before and during pregnancy does not cause negative effects on the foetus or the mother.

- Betamethasone appears to be a potential option for gestational protection even in patients without a history of pregnancy loss. In addition to women undergoing in vitro fertilization and embryo transfer, even women in advanced age, at their first apparently normal spontaneous pregnancy, are candidates for betamethasone protection, simply because many of them will not be given a second chance to become mothers.

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