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Placental vascular pathology and increased thrombin generation as mechanisms of disease in obstetrical syndromes

Obstetrical complications including preeclampsia, fetal growth restriction, preterm labor, preterm prelabor rupture of membranes and fetal demise are all the clinical endpoint of several underlying mechanisms (i.e. infection inflammation, thrombosis, endocrine disorder, immunologic rejection, genetic, and environmental), therefore, they may be regarded as syndromes. Placental vascular pathology and increased thrombin generation were reported in all of these obstetrical syndromes. Moreover, elevated concentrations of thrombin-anti-thrombin III complexes and changes in the coagulation as well as anticoagulation factors can be detected in the maternal circulation prior to the clinical development of the disease in some of these syndromes. In this review, we will assess the changes in the hemostatic system during normal and complicated pregnancy in maternal blood, maternal-fetal interface and amniotic fluid, and describe the contribution of thrombosis and vascular pathology to the development of the great obstetrical syndromes.

1 **Placental Vascular Pathology and Increased Thrombin Generation as Mechanisms of**
2 **Disease In Obstetrical Syndromes**

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20 **1. Introduction**

21 Obstetrical complications including preeclampsia, fetal growth restriction, preterm labor, preterm
22 prelabor rupture of membranes and fetal demise are all the clinical endpoint of several underlying
23 mechanisms (i.e. infection inflammation, thrombosis, endocrine disorder, immunologic rejection,
24 genetic, and environmental), therefore, they may be regarded as syndromes. In this review, we
25 will assess the changes in the hemostatic system during normal and complicated pregnancy in
26 maternal blood, maternal-fetal interface and amniotic fluid, and describe the contribution of
27 thrombosis and vascular pathology to the development of the great obstetrical syndromes.

28 **2. What are the great obstetrical syndromes?**

29 The major obstetrical complications including preeclampsia, intrauterine growth restriction
30 (IUGR), preterm labor (PTL), preterm prelabor rupture of membranes (PROM), fetal demise, and
31 recurrent abortions are all syndromes, also defined as "great obstetrical syndromes". As reported
32 in The Oxford Medical Dictionary a syndrome is 'a combination of symptoms and/or signs that
33 form a distinct clinical picture indicative of a particular disorder'. Hence, they represent the
34 clinical manifestation of many possible underlying mechanisms of disease¹.
35 Key features of these syndromes are²: multiple etiologies; long preclinical stage; frequent fetal
36 involvement; clinical manifestations which are often adaptive in nature; and predisposition to a
37 particular syndrome is influenced by gene–environment interaction and/or complex gene-gene
38 interactions involving maternal and/or fetal genotypes. These mechanisms of disease were
39 identified and reported in all the obstetrical complications listed above. This review is focused on
40 the role of thrombosis and vascular pathology of the placenta in these syndromes.

41 **3. What are the changes in the coagulation system during normal pregnancy?**

42 In term of the coagulation and hemostatic systems there are several major compartments: the
43 maternal circulation, the fetal maternal interface (the placenta, and membranes), amniotic fluid
44 and the fetus that each has a specific behavior during gestation. The changes in the coagulation
45 system during gestation are considered to be adaptive mechanisms and are aimed to: 1) the
46 prevention of bleeding at the time of trophoblast implantation and the delivery of the fetus; 2)
47 allow the laminar flow and the intervillous space; and 3) seal amniotic fluid leak and reduce
48 obstetrical bleeding³⁻⁷. Of interest, the fetus is somewhat less involved and its coagulation system
49 develops during gestation, and this subject is beyond the scope of this review.
50 Indeed, normal pregnancy has been associated with excessive maternal thrombin generation^{3, 8}
51 and a tendency for platelets to aggregate in response to agonists^{9, 10}. Pregnancy is accompanied by
52 2 to 3-fold increase in fibrinogen concentrations and 20% to 1000% increase in factors VII, VIII,
53 IX, X, and XII, all of which peak at term¹¹. The concentrations of vWF increase up to 400% by
54 term¹¹. By contrast, those of pro-thrombin and factor V remain unchanged while the
55 concentrations of factors XIII and XI decline modestly¹². Indeed there is evidence of chronic low-
56 level thrombin and fibrin generation throughout normal pregnancy as indicated by enhanced
57 concentrations of pro-thrombin fragment 1.2, thrombin-antithrombin (TAT) III complexes, and
58 soluble fibrin polymers¹³. Free protein S concentration declines significantly (up to 55%) during
59 pregnancy due to increased circulating complement 4B-binding protein its molecular carrier.
60 Protein S nadir at delivery and this reduction is exacerbated by cesarean delivery and infection¹¹.
61 ¹². As a consequence, pregnancy is associated with an increase in resistance to activated protein

62 C^{12, 13}. The concentrations of PAI-1 increase by 3 to 4-folds during pregnancy while plasma PAI-2
63 values, which are negligible before pregnancy reach concentrations of 160 mg/L at delivery¹¹.
64 Thus, pregnancy is associated with increased clotting potential, as well as decreased
65 anticoagulant properties, and fibrinolysis¹⁴. Therefore, it can be defined as a prothrombotic state.
66 One of the most important mediators of the hypercoagulable state of normal pregnancy is tissue
67 factor. Indeed, there is a substantial increase in tissue factor (TF) concentrations in the decidua
68 and myometrium¹⁵⁻¹⁸, as well as preventing placental abruption. The placenta is a source of TF,
69 since trophoblast cells constitutively express it, behaving as activated endothelium, and leading to
70 a condition of procoagulant state that, if not controlled by anticoagulant mechanisms, predisposes
71 to thrombotic complications¹⁵. The principal anticoagulant mechanism inhibiting TF activation
72 pathway is tissue factor pathway inhibitor (TFPI), which mRNA is highly expressed in the
73 macrophages in the villi in term placenta¹⁹.
74 Similarly, high TF concentrations have been detected in the fetal membranes (mainly the amnion)
75 and amniotic fluid^{7, 20-23}. TFPI has been found in amniotic fluid as well²⁰, but it is not clear if the
76 presence of TF and its natural inhibitor is related to coagulation per se or is somehow connected
77 with embryonic development²⁴.
78 In contrast to the changes detected in the amniotic fluid and the decidua, the median maternal
79 plasma immunoreactive TF concentration of normal pregnant women do not differ significantly
80 from that of non-pregnant patients^{3, 25}. However, labor at term increases significantly the maternal
81 plasma immunoreactive TF concentration in comparison to the non-pregnant state²⁰. In addition
82 to the changes in TF, normal pregnancy is associated with increased thrombin generation^{3, 8}, as
83 determined by the elevation of maternal concentrations of fibrinopeptide A, prothrombin
84 fragments (PF) 1 and 2, and thrombin–antithrombin (TAT) III complexes^{7, 26-28}. The concentration
85 of these complexes further increases during and after normal parturition^{27, 29}, and subsequently
86 decreases during the puerperium^{27, 29}.

87 **4. What are the changes in the hemostatic system associated with the great obstetrical** 88 **syndrome?**

89 The great obstetrical syndromes are associated with changes in the hemostatic and vascular
90 systems in the compartments mentioned above: 1) the maternal circulation; 2) the fetomaternal
91 interface of placenta and membranes; 3) and the amniotic fluid.

92 **4.1 Changes in the hemostatic system of women with obstetrical syndromes.**

93 The involvement of the hemostatic system in the pathophysiology of these obstetrical syndromes
94 is becoming more and more apparent. Indeed, increased thrombin generation is reported in the

95 maternal circulation of women with preeclampsia³⁰⁻³⁴, IUGR^{30-32, 35, 36}, fetal demise³⁷, PTL^{8, 37, 38} and
96 preterm PROM^{8, 37, 39}.
97 There are several possible explanations for the increased thrombin generation in these patients: 1)
98 increased activation of coagulation cascade in the maternal circulation due to pathological
99 processes including bleeding or inflammation; and 2) depletion of anticoagulation proteins that
100 subsequently leads to increased thrombin generation (Table 1).

101 **4.1.1 Increased activation of the coagulation cascade and thrombin generation in the** 102 **maternal circulation in patients with pregnancy complications**

103 All the obstetrical syndromes including preeclampsia^{30-34, 40, 41}, FGR^{31, 32, 35, 36}, fetal demise³⁷, PTL^{8,}
104 ³⁸ and preterm PROM^{8, 37, 39} are associated with a higher maternal thrombin generation than a
105 normal pregnancy. These may be of clinical implication since in women with preterm labor,
106 elevated maternal plasma TAT III complexes concentration was associated with a higher chance
107 to deliver within <7 days from admission³⁷ (Fig. 1). To further understand how does thrombin
108 affect the duration of pregnancy and the clinical phenotype of patients with the obstetrical
109 syndromes we need to consider what are the mechanisms leading to thrombin generation and how
110 it affects the fetomaternal unit.

111 Increased thrombin generation can result from the following underlying mechanisms: 1) decidual
112 hemorrhage that leads to a retro-placental clot formation⁴²; 2) intra-amniotic
113 infection/inflammation which can induce decidual bleeding and sub-clinical abruption⁴³, as well
114 as increased intra-amniotic TAT complexes³⁷; and 3) an increased maternal systemic
115 inflammatory response⁴⁴ that may activate the extrinsic pathway of coagulation due to the
116 expression and release of TF by activated monocytes⁴⁵.

117 Thrombin affects many systems including also the following: 1) stimulation of decidual cell
118 secretion of matrix metalloproteinase (MMP) (i.e. MMP-1 and MMP-3) that can degrade the
119 extracellular matrix of the chorioamniotic membranes^{46, 47} (as in preterm PROM); 2) myometrial
120 activation and uterine contractions generation that may lead to preterm labor with or without
121 rupture of membranes and a subsequent preterm delivery^{38, 48, 49}; and 3) thrombin has an inhibitory
122 effect on the production of TFPI by endothelial cells⁵⁰, and the increased thrombin generation
123 observed in patients with PTL may be associated with a concomitant reduction in TFPI
124 production by the maternal vascular endothelium (the depletion of anticoagulant proteins will be
125 discussed in the following section of this review).

126 There is evidence to support that the extrinsic pathway of coagulation is activated in many of
127 these pregnancy complications and it is the source of the increased thrombin generation⁵¹. Indeed,
128 increased immunoreactive TF concentrations were reported in women with preeclampsia and

129 those with preterm PROM⁵². Moreover, the contribution of preeclampsia to elevated maternal
130 immunoreactive TF persisted also among patients with fetal demise, while those with fetal death
131 who were normotensive did not have higher median TF concentration than normal pregnant
132 women. Moreover, the median TF concentration of patients with preeclampsia was also higher
133 than in patients with fetal demise without hypertension. These findings are consistent with
134 previous studies^{3, 53}, suggesting that elevated TF immunoreactivity and activity may be associated
135 with the pathophysiologic process leading to preeclampsia, rather than being a consequence of
136 the fetal death.

137 In some of the obstetrical syndromes there was elevated tissue factor activity in the maternal
138 circulation without a concomitant increase in the plasma concentration of this factor. This was the
139 case among patients with an SGA neonate and those with preterm labor^{54 31} (Table 1). This
140 suggests that the increased TF activity among patients with PTL as well as those with an SGA
141 neonate, contributes to a higher generation of factor Xa that, along with the physiologic increase
142 in the maternal plasma concentrations of factor VII and factor X during gestation^{11, 55-57}, may be
143 the underlying mechanism leading to the increased thrombin generation reported these
144 syndromes.

145 The differences between PTL and preterm PROM in term of maternal plasma TF concentration
146 and activity may derive from the specific component of the common pathway of parturition,
147 which is activated in each obstetrical syndrome⁵⁸. While preterm PROM is associated with the
148 activation of the decidua and the membranes, myometrial activation is the major component of
149 preterm labor with intact membranes⁵⁸. This is relevant because the decidua and the membranes
150 have a high TF concentration^{17, 18, 59}.

151 In summary, the evidence brought herein suggests that increased thrombin generation in patients
152 with the great obstetrical syndromes may reflect the activation of the coagulation cascade mainly
153 through the extrinsic arm. This activation can be attributed to various underlying mechanisms.

154 **4.1.2 Depleted or insufficient anticoagulant proteins concentration**

155 In the normal state there is a delicate balance between the proteins activating/participating the
156 coagulation cascade and their inhibitors. Increased thrombin generation may result, as we
157 presented above, from activation of the coagulation cascade due to higher concentrations or
158 activities of the proteins included in the coagulation cascade. However, thrombin generation can
159 also result from insufficient concentration or activity of anticoagulation proteins.

160 Tissue factor pathway inhibitor (TFPI), a glycoprotein comprising of three Kunitz domain⁶⁰ that
161 are specific inhibitors of trypsin-like proteinases⁶¹, is the main inhibitor of the extrinsic pathway
162 of coagulation. TFPI inhibits thrombin generation through the inactivation of activated factor X

163 and the factor VIIa/TF complex^{60, 62}. The mean maternal plasma concentrations of total TFPI
164 increases during the first half of pregnancy, remains relatively constant in the second half⁶³ and
165 decreases during labor²⁰. There are two types of TFPI: 1) TFPI-1 is the more prevalent form in
166 the non-pregnant state in the maternal circulation and can also be found in the fetal blood,
167 platelets, endothelial cells and other organs^{19, 64}; and 2) TFPI-2- the major form of TFPI in the
168 placenta⁶⁵⁻⁶⁸, also known as Placental Protein 5 (PP5)^{69, 70}. During pregnancy, the maternal plasma
169 concentration of TFPI-2 increases gradually, reaches a plateau at 36 weeks and subsides after
170 delivery⁷¹⁻⁷⁴.

171 The overall balance between the concentration and activity of the coagulation factors and the
172 anti-coagulation proteins is one of the determining factors of thrombin generation. In the normal
173 state, the immunoreactive concentrations of TFPI in the plasma are 500 to 1000 times higher than
174 that of TF⁷⁵, suggesting that an excess of anti-coagulant proteins closely controls the coagulation
175 cascade activity. The median maternal plasma TFPI concentration increases during
176 preeclampsia^{53, 76}, which is associated with an exaggerated maternal systemic inflammatory
177 response. However, the increase in the median maternal TF plasma concentration is such that the
178 overall balance between TF and its inhibitor is affected leading to increased thrombin generation
179 in this syndrome. In contrast to preeclampsia, maternal plasma TFPI concentration decreases in
180 patients with PTL⁵² and preterm PROM⁷⁷ regardless to the presence of intra-amniotic
181 infection/inflammation, as well as in women with fetal demise⁵⁴, and does not change in mothers
182 with SGA fetuses⁵³. Overall these findings suggest that the increased thrombin generation
183 observed among these patients may derive not only from an increased activation of the
184 hemostatic system, but also from insufficient anti-coagulation, as reflected by the lower TFPI
185 concentrations (Fig. 2).

186 A possible explanation of the lower maternal plasma concentration observed in some of the
187 obstetrical syndromes may be that during these syndromes there is a reduction in the placental
188 production of TFPI^{65, 66, 69, 76} (mainly TFPI-2), contributing to the low maternal plasma
189 concentrations detected in patients with PTL, in addition to the thrombin inhibitory effect to TFPI
190 expression on endothelial cells, as above mentioned. Indeed, patients with vascular complications
191 of pregnancy (preeclampsia, eclampsia, placental abruption, fetal growth restriction, and fetal
192 demise) have a lower placental concentration of total TFPI, and TFPI mRNA expression than in
193 women with normal pregnancies^{78, 79}.

194 Other proteins implicated in the inhibitory control of the coagulation cascade are protein S,
195 protein C and protein Z. Protein S is a cofactor to protein C in the inactivation of factors
196 Va and VIIIa. This protein exists in two forms: a free form and a complex form bound

197 to complement protein C4b-binding protein (C4BP). Only the free form is active⁸⁰. Protein S also
198 acts as a TFPI cofactor, in the presence of weak pro-coagulant stimuli, by enhancing the
199 interaction of TFPI with factor Xa while using Ca²⁺ and phospholipids in the process⁸¹ without
200 increasing inhibition of factor VIIa-TF by TFPI⁸². During pregnancy there is a physiologic
201 change in the relationship between the bound and the free forms of protein S in the maternal
202 plasma. The increase in C4BP during gestation reduces free protein S concentration in up to 55%
203 of its value out of pregnant state, reaching its nadir at delivery. Of interest, cesarean delivery and
204 infection exacerbate the reduction in free protein S concentrations^{11, 83}. Moreover, a functional
205 protein S deficiency can explain a poor response to activated protein C⁸⁴.
206 The association between the alteration of concentration and function of protein S and protein C in
207 the great obstetrical syndromes is not completely clear. The evidence regarding the association of
208 protein S and protein C deficiency and preeclampsia is controversial^{85, 86}.
209 While some reported an association between protein S deficiency and an increased risk for this
210 syndrome (especially for early onset preeclampsia)⁸⁵ others could not demonstrate this effect⁸⁶.
211 There is some evidence regarding the relation of protein S deficiency and increased risk of
212 stillbirth⁸⁷ and mid-trimester IUGR⁸⁸. An increased risk of stillbirth has been reported in patients
213 with protein S deficiency while the risk was not significantly increased in cases of protein C
214 deficiency⁸⁷, and Kupferminc et al⁸⁸ found that protein S, but not protein C deficiency, was
215 significantly associated with severe mid-trimester IUGR.
216 Protein Z, in complex with protein Z-dependent protease inhibitor (ZPI) (Fig. 3)⁸⁹⁻⁹¹, acts as a
217 physiologic inhibitor of activation of prothrombin by factor Xa. Protein Z is a vitamin
218 K-dependent plasma glycoprotein⁹² that is an essential cofactor for ZPI activity. In the absence of
219 protein Z, the activity of ZPI is reduced by more than 1000-fold⁹¹. Normal pregnancy is
220 characterized by an increased plasma concentration of protein Z⁹³, probably as a compensation
221 for the increase of factor X concentration. Women with preterm labor without intra-amniotic
222 infection or inflammation and those with vaginal bleeding who delivered preterm had a lower
223 median maternal plasma protein Z concentration than women with a normal pregnancy and those
224 with vaginal bleeding who delivered at term⁹⁴. The changes of protein Z concentrations in other
225 pregnancy complications are controversial. Some demonstrated that the median plasma
226 concentration of protein Z in patients with preeclampsia, IUGR, and late fetal death were not
227 significantly different than that of patients with a normal pregnancy⁹⁵. Others reported lower
228 median maternal plasma protein Z concentrations in women with preeclampsia or pyelonephritis
229 and higher proportion of protein Z deficiency (defined as protein Z plasma concentration below
230 the 5th percentile) in patients with preeclampsia or fetal demise than in those with a normal

231 pregnancy⁹⁶. Moreover, increased maternal plasma anti-protein Z antibodies concentrations were
232 associated with SGA neonates, fetal demise and preeclampsia.
233 The information presented above suggest that it is not only the concentration of one coagulation
234 factor or anticoagulation protein, but rather the overall balance between the coagulation factors
235 and their inhibitors that increases thrombin generation in the great obstetrical syndromes. Indeed,
236 although preterm labor was not associated with a significant change in the median maternal
237 plasma TF concentration, the TFPI/TF ratio of these patients was lower than that of normal
238 pregnant women, mainly due to decreased TFPI concentrations.
239 This observation was also reported in patients with preterm PROM⁷⁷, and those with
240 preeclampsia⁵³. The lower TFPI/TF ratio in patients with preeclampsia occurs despite the increase
241 in the median maternal plasma TFPI concentration observed in these patients. This suggests that
242 the balance between TF and its natural inhibitor may better reflect the overall activity of the TF
243 pathway of coagulation, than the individual concentrations of TF or TFPI.
244 Collectively, these observations suggest that our attention should be focused not only on the
245 coagulation protein but also on their inhibitors since an imbalance between them may contribute
246 to increased thrombin generation leading to the onset of the great obstetrical syndromes.

247 **4.2 Changes in the feto-maternal interface**

248 Normal placental development and the establishment of an adequate feto-maternal circulation are
249 key points for a successful pregnancy. The networks of the placental vascular tree either on the
250 maternal or fetal side are dynamic structures that can be substantially altered in cases of abnormal
251 placentation and trophoblast invasion. The human trophoblast has properties of endothelial cells
252 and can regulate the degree of activation of the coagulation cascade in the intervillous space^{97, 98}.
253 The vilous trophoblasts express heparin sulfate, protein C and protein Z on their surface that
254 serve as anticoagulant that sustain laminar blood flow through the intervillous space. On the other
255 hand, unlike the endothelium of other organs, the trophoblast constantly presents the active
256 placental isoform of TF on its surface⁹⁸⁻¹⁰¹. This isoform has a higher affinity for factor VIIa¹⁰²,
257 which may lead to increased activation of the coagulation cascade. One of the leading
258 pathological processes observed in all these syndromes is thrombosis and vascular abnormality of
259 the placenta at the maternal-fetal interface. The incidence of these pathological processes varies
260 among the different syndromes being more prevalent in preeclampsia, IUGR, and fetal demise
261 than in PTL and preterm PROM^{30, 31, 37, 38}.

262 **4.2.1 Placental pathology in the Great Obstetrical Syndromes**

263 There is a range of placental vascular and thrombotic lesions that are being observed in placentas
264 of patients with pregnancy complications. Thrombotic events of placental vessels can cause an
265 impairment of placental perfusion, leading to FGR, preeclampsia and fetal death as well as in
266 some extents to PTL and preterm PROM^{103, 104}. The frequency of the specific vascular placental
267 lesions varies among these obstetrical syndromes¹⁰⁵.

268 Placental vascular lesions are divided into maternal or fetal vascular origin (figure 1-2)^{106, 107}.
269 Lesions of the maternal vascular compartment include placental marginal and retro-placental
270 hemorrhages, lesions related to maternal under perfusion (acute atherosclerosis and mural hypertrophy,
271 increased syncytial knots, villous agglutination, increased intervillous fibrin deposition, villous
272 infarcts)¹⁰⁶. Placental fetal vascular obstructive lesions are the result of stasis, hypercoagulability
273 and vascular damage within the fetal circulation of the placenta. Placental fetal vascular
274 abnormalities include: cord-related abnormalities (as torsion of cord, over-coiling, strictures and
275 tight knots¹⁰⁸) and vascular lesions consistent with fetal thrombo-occlusive disease (thrombosis of
276 the chorionic plate and stem villous vessels, fibrotic, hypo-vascular and avascular villi¹⁰⁶. In
277 addition, villitis of unknown etiology or chronic villitis, defined as lymphohistiocytic

278 inflammation localized to the stroma of terminal villi but often extending to the small vessels of
279 upstream villi is also associated with obliterative fetal vasculopathy¹⁰⁶ (Fig. 4-5).

280 **Preeclampsia:** The classical example for an association between obstetrical syndrome and
281 vascular placental lesions is preeclampsia. Women who develop preeclampsia have an increased
282 rate of abnormalities of the maternal side of the placental circulation and maternal
283 underperfusion^{109, 110}. The frequency of these lesions is inversely related to the gestational age in
284 which the hypertensive disorder was diagnosed. The earlier the development of
285 hypertension/preeclampsia the more severe are the vascular lesions^{111, 112}. Moreover, Kovo et al¹¹³
286 reported that the presence of fetal growth restriction in women with preeclampsia increases also
287 the frequency of fetal vascular lesions. Indeed, patients with early-onset preeclampsia
288 complicated by FGR had a higher rate of fetal-vascular supply lesions consistent with fetal
289 thrombo-occlusive disease than women with early-onset disease without FGR¹¹³.
290 An assessment of the pathologic changes in placental hemostatic system has been performed in
291 patients with preeclampsia. Teng et al¹¹⁴ studied TF and TFPI placental levels in pregnant patients
292 with preeclampsia, compared to normal pregnancies. They found increased TF placental
293 expression and a reduced expression of TFPI-1 and TFPI-2, with a significant correlation
294 between the levels of TF and TFPI-2 between maternal plasma and placenta.

295 **Fetal growth restriction:** Placentas from pregnancies complicated by FGR are smaller and have
296 significantly increased maternal and fetal vascular lesions compared to placentas from normal
297 pregnancies with appropriate for gestational age neonates (AGA)^{115, 116}. Maternal vascular
298 lesions were detected in about 50% of placentas from pregnancies complicated with FGR at term,
299 compared to only 20% in normal pregnancies, while fetal vascular lesions were observed in 11%
300 of FGR pregnancies compared to only 4% in placentas from normal pregnancies¹¹³.
301 Placentas from normotensive pregnancies complicated by early-onset FGR (<34 weeks of
302 gestation) had a higher rate of low placental weight (<10th percentile) and maternal
303 underperfusion, as compared to placentas of women who delivered AGA neonates \leq 34 weeks of
304 gestation¹¹⁵. Of interest, placentas from the late onset FGR group (after 34 weeks of gestation), in
305 addition to the high maternal vascular abnormalities, show also more fetal vascular abnormalities,
306 compared with AGA controls who delivered >34 weeks¹¹⁷.

307 **Fetal demise:** Placental disease has been recognized as an important contributor to unexplained
308 fetal demise. Fetal vascular abnormalities¹⁰⁵ are extensively involved in early and late fetal death
309 rather than maternal vascular lesions. In fetal death occurring prior to 34 weeks, an earlier and

310 extended insult in the placental development occurs. On the other hand, late fetal demise is an
311 unpredicted event that is mostly characterized by non-thrombotic cord related lesions and less
312 placental vascular compromise¹⁰⁷.

313 **Preterm labor and preterm PROM:** Placental studies in PTL demonstrated a combination of
314 inflammatory and vascular lesions. PTL is generally attributed to an inflammatory response
315 involving the bacterial induction of cytokine and prostanoid production¹¹⁸. Finding of histological
316 chorioamnionitis in PTL¹¹⁹ has established infection and inflammation as a causative factor of
317 preterm birth, moreover, noninfectious trigger may also contribute to the development of preterm
318 labor and in some instances may be evident by placental sterile inflammatory response¹²⁰. In
319 addition, isolated placental vascular lesions, mostly of maternal supply, were reported in 20% of
320 cases of PTL and an additional 20% had combined inflammatory and vascular lesions. Moreover,
321 there are consistent reports describing increased rate of failure of transformation of the spiral
322 arteries in women with preterm labor without intrauterine infection/inflammation and in those
323 with preterm PROM than in women with normal pregnancies¹²¹. Such findings imply that an
324 inadequate uteroplacental blood flow due to abnormal placentation plays an important role in
325 pathogenesis of preterm parturition^{121, 122}.

326
327 Collectively, placental vascular lesions were reported in all the great obstetrical syndromes. The
328 severity of these lesions is associated with the timing of diagnosis of the disease. The more severe
329 the vascular injury, the more likely these complications will become clinically evident prior to 34
330 weeks of gestation. Of interest, vascular lesions often come along with evidence of acute
331 inflammation or lesions associated with chronic inflammatory processes, suggesting that
332 sometimes more than one mechanism is involved in development of a specific obstetrical
333 syndrome.

334 **4.3. Hemostatic changes in the amniotic fluid of women with obstetrical syndromes**

335 During normal pregnancy, there is an increase in the amniotic fluid TF concentration^{7, 20-23}. In
336 order to demonstrate the association of hemostatic changes and the development of obstetrical
337 complications, Erez et al⁵⁴ studied the changes in the intra-amniotic concentration of TAT III
338 complexes, as well as TF concentration and activity, in cases of fetal demise and in normal
339 pregnancies.

340 Patients with a fetal demise had higher median amniotic fluid–TF concentration and activity than
341 those with normal pregnancies. Moreover, among patients with a FD there was a significant
342 correlation (Fig. 6) between the amniotic fluid–TF concentrations and activity ($r = 0.88$, P

343 <0.0001). The median amniotic fluid– TAT III complexes concentration did not differ
344 significantly between the groups (normal pregnancy: median: 66.3 mg/l, range 11.4–2265.4 vs.
345 FD: median: 59.3 mg/l, range: 13.6–15,425.3; P =0.7). In their study, the median amniotic fluid–
346 TF concentration in normal pregnant women was 10 fold higher than in maternal plasma.
347 The changes in amniotic fluid thrombin generation were reported also in women with preterm
348 parturition. Indeed, intra-amniotic infection and/or inflammation is associated with an increased
349 amniotic fluid TAT III complexes (Fig. 7). This is important since it represents an increased
350 thrombin generation in the amniotic cavity during infection and/or inflammation that may
351 contribute to uterine contractility and the development of preterm birth¹²³. Of interest, elevated
352 intra-amniotic TAT III concentrations were associated with a shorter amniocentesis to delivery
353 interval and an earlier gestational age at delivery only in patients with preterm labor without
354 intra-amniotic infection or inflammation¹²³. This observation suggests that in a subset of patients
355 with preterm labor, activation of the coagulation system can generate preterm parturition and
356 delivery; while in those with intra-amniotic infection and/or inflammation the activation of the
357 coagulation and thrombin generation is a byproduct of the inflammatory process leading to
358 preterm birth.
359 This represents evidence of the activation and propagation of coagulation cascade, being
360 thrombin generation the witness of the former mechanisms and the inhibitor of the initiation
361 step⁵⁴.

362 **5. Conclusion**

363 The evidence presented herein suggests a role for increased thrombin generation and vascular
364 placental lesions in the pathogenesis of the great obstetrical syndromes. This process can be the
365 result of the contribution of procoagulant and vascular abnormalities as well as inflammatory and
366 infectious mechanisms, representing the starting point for pregnancy complications based on
367 vascular disease.
368 As presented, these changes affect the mother, the placenta, membranes and amniotic fluid.
369 Moreover, preliminary evidence suggest that some of the changes in the hemostatic system in the
370 mother and in the amniotic fluid predate the clinical presentation of the disease. Suggesting that
371 better understanding of the vascular and coagulation changes associated with the great obstetrical
372 syndromes may assist us in earlier detection and the development or introduction of therapeutic
373 modalities for these syndromes.

374

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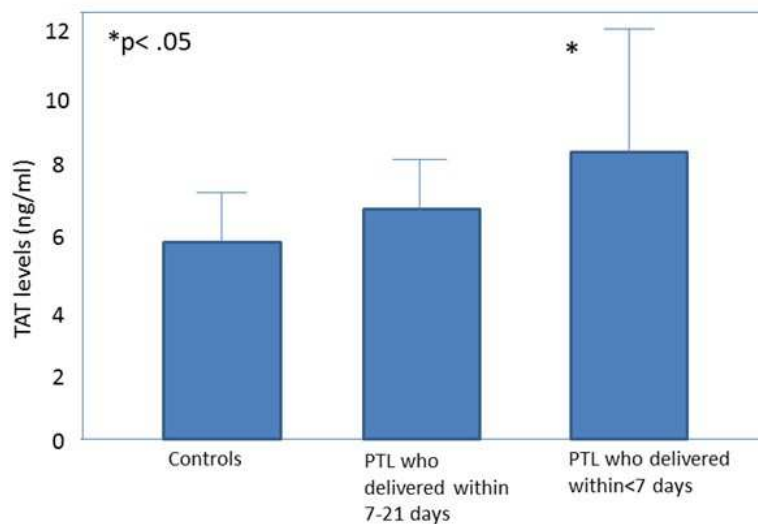
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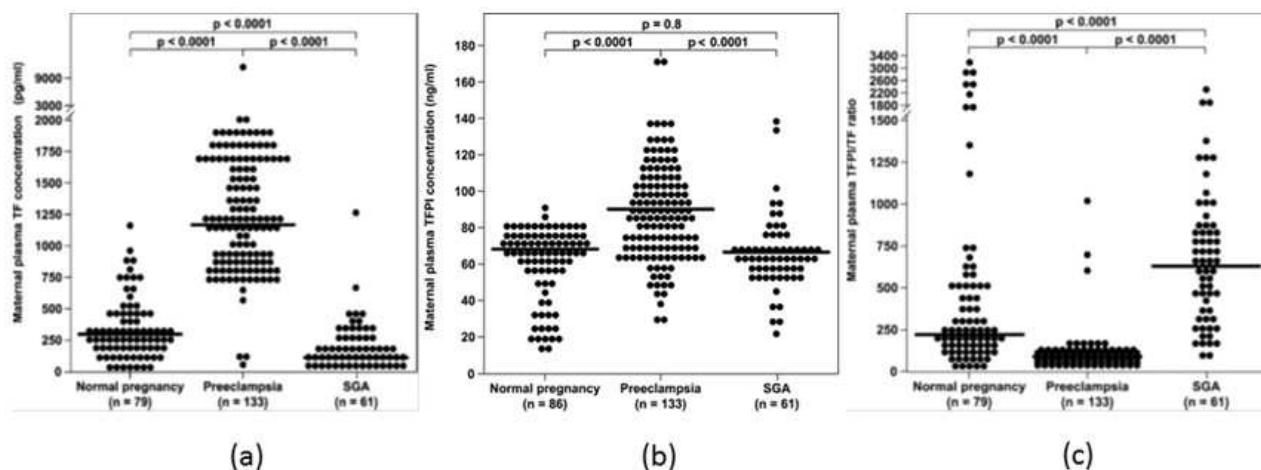
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654 Figure 1. Thrombin–antithrombin III (TAT) levels in control patients, patients with preterm labor
 655 who delivered within 3 weeks, and patients with preterm labor who delivered after 3 weeks. Open
 656 diamonds, Mean levels; black error bars, SD. *P <.05, Student-Newman-Keuls method (from
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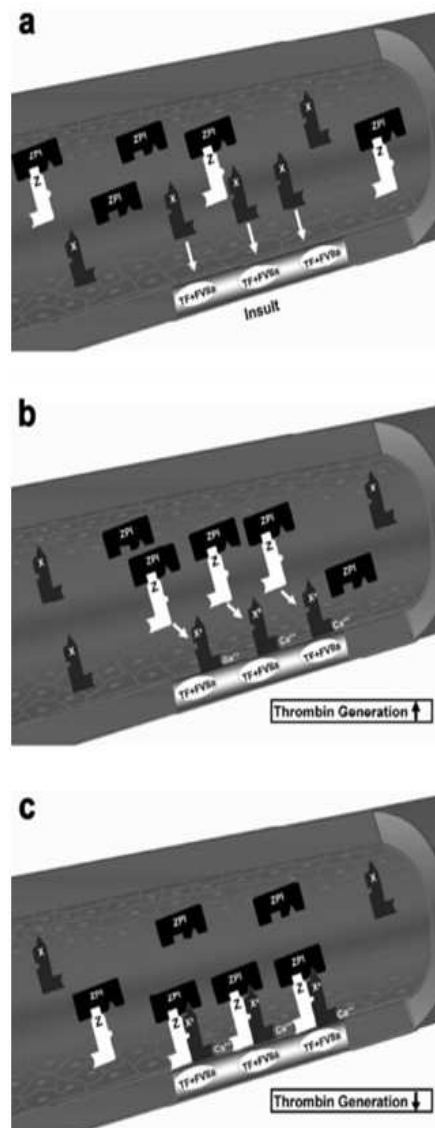


659 Figure 2. (a) Comparison of median maternal plasma TF concentration between patients with
 660 normal pregnancy (n=79), pre-eclampsia (n=133), and women who delivered an SGAneonate
 661 (n=61). (b) Comparison of median maternal plasma TFPI concentration between patients with
 662 normal pregnancy (n=86), pre-eclampsia (n=133), and women who delivered an SGA neonate

663 (n=61). (c) Comparison of maternal plasma TFPI/TF ratio between women with normal
 664 pregnancy (n=79), pre-eclampsia (n=133), and women who delivered an SGA neonate (n=61).
 665 (From [Erez O, Romero R, Hoppensteadt D, Than NG, Fareed J, Mazaki-Tovi S, Espinoza](#)
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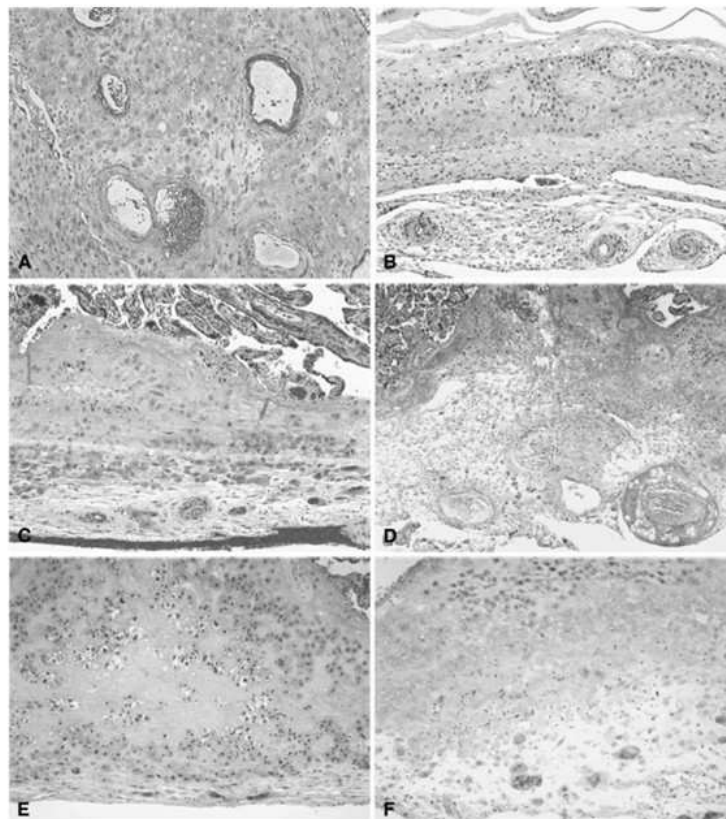


669 Figure 3. Factor X activation and protein Z/protein Z-dependent protease inhibitor (ZPI)
 670 inhibition of activated factor X. (a) Then formation of the complex of tissue factor (TF) and
 671 factor VIIa (FVIIa) at the site of injury and activation of extrinsic coagulation cascade. (b)
 672 Activation of circulating factor X by the TFpFVIIa complex in the presence of exposed
 673 phospholipids and Ca²⁺. (c) Inhibition of factor Xa (FXa) by the protein Z/ZPI complex by
 674 binding to its active site. Modified from Broze JG, Lancet 2001;357:900–901.

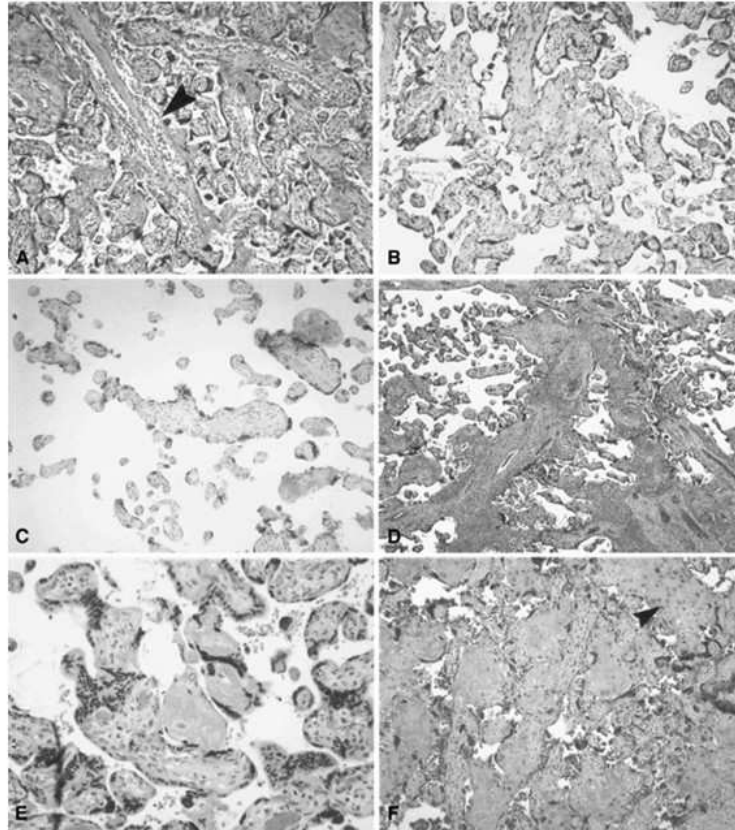


675 Figure 4. Histologic features of maternal vessel and implantation site reaction patterns: a. Acute
 676 atherosclerosis of maternal arterioles in the placental membranes: a cluster of decidual arterioles shows
 677 varying stages of fibrinoid necrosis. The vessel at the upper right shows full histologic expression
 678 with dark homogenous fibrinoid replacement of the vessel wall accompanied by occasional
 679 foamy macrophages ([original magnification is indicated for all panels] X 20). b. Mural
 680 hypertrophy of decidual arterioles in the placental membranes: a cluster of arterioles shows
 681 medial hypertrophy with the vessel wall occupying greater than one third of total vessel diameter
 682 (X 10). c. Muscularized basal plate arteries with accompanying implantation site abnormalities:
 683 maternal spiral arteries in the basal plate lack normal trophoblast remodeling and retain their pre-

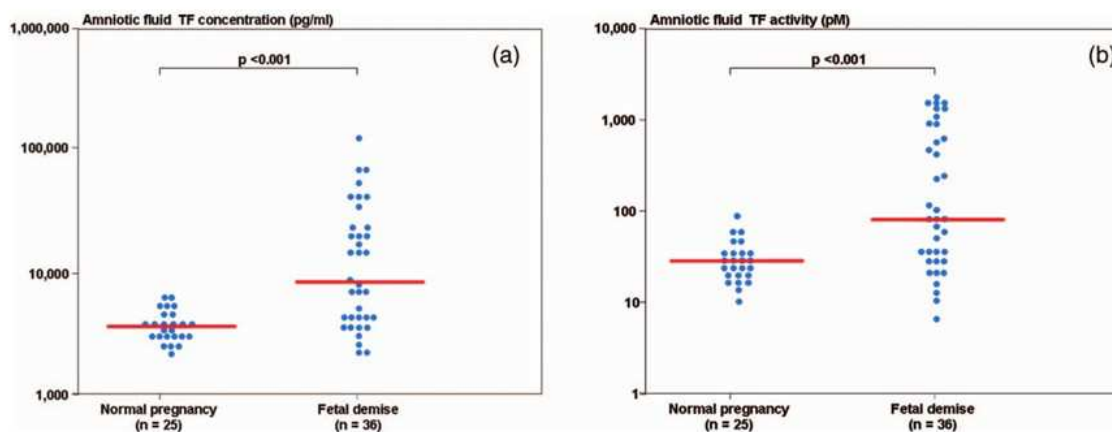
684 pregnancy muscular media. Clusters of immature intermediate trophoblast and increased
685 placental giant cells are seen above and below the muscular arteries, respectively (X 10). d. Acute
686 atherosclerosis of muscularized basal plate arteries with accompanying implantation site abnormalities:
687 three cross sections of a basal plate artery are seen. The two on the left show persistence of the
688 muscular media while the one on the right has undergone fibrinoid necrosis of the media with
689 foamy macrophages (acute atherosclerosis). Clusters of immature intermediate trophoblast are also
690 seen overlying the arteries (X 4). e. Immature intermediate trophoblast: clusters of abnormally
691 small intermediate trophoblast with focal vacuolation are surrounded by an excessive amount of
692 basal plate fibrin. Increased placental site giant cells are also seen at the lower margin (X 10). f.
693 Increased placental site giant cells: numerous multinucleate placental site giant cells, not usually
694 seen in the delivered placenta, are scattered in loose decidual tissue which is devoid of normal
695 intermediate trophoblast and fibrinoid (X 10).



696 Figure 5. Histologic features of villous and intervillous lesions; a. Increased syncytial knots:
697 aggregates of syncytiotrophoblast nuclei cluster at one or more poles of distal villi in the vicinity
698 of larger stem villi (arrowhead) at the periphery of the lobule ([original magnification is indicated
699 for all panels] X 10). b. Villous agglutination: clusters of degenerating distal villi are adherent to
700 one another and focally enmeshed in fibrin (X 4). c. Distal villous hypoplasia: a long, thin, non-
701 branching stem villus is surrounded by a markedly reduced number of small hypoplastic distal
702 villi (X 10). d. Increased intervillous fibrin: stem villi are surrounded by a mantle of fibrin-type
703 fibrinoid that does not extend to distal villi at the center of the lobule (X 2). e. Nodular
704 intervillous (and intravillous) fibrin: small aggregates of intervillous fibrin adhere to, and are
705 focally reepithelialized by, distal villous trophoblast (X 20). f. Increased intervillous fibrin with
706 intermediate trophoblast (X-cells): stem and distal villi are enmeshed in a matrix of fibrin and
707 fibrinoid containing prominent intermediate trophoblast (arrowhead) (X 10).



708 Figure 6. Amniotic fluid tissue factor concentration among women with normal pregnancies
709 (median 3710.4 pg/ml, range 2198.8–6268) and patients with a fetal demise (median 8535.4
710 pg/ml, range 2208.2–125,990.0); (b) Amniotic fluid tissue factor activity among women with
711 normal pregnancies (median 28.4 pM, range 10.2–84.9) and patients with a fetal demise (median
712 81.6 pM, range 7.2–1603.4). From EREZ O, GOTSCH F, MAZAKI-TOVI S, et al. Evidence of
713 maternal platelet activation, excessive thrombin generation, and high amniotic fluid tissue factor
714 immunoreactivity and functional activity in patients with fetal death. *J Matern Fetal Neonatal*
715 *Med* 2009;22:672-87, with permission.



716 Figure 7. Maternal plasma TAT III concentration in women with preterm labor (PTL) and those
717 with a Normal pregnancy (From Chaiworapongsa T, Espinoza J, Yoshimatsu J, Kim YM, Bujold
718 E, Edwin S, et al.
719 Activation of coagulation system in preterm labor and preterm premature rupture of membranes.
720 J Matern Fetal Neonatal Med 2002 11(6):368-373, with permission)

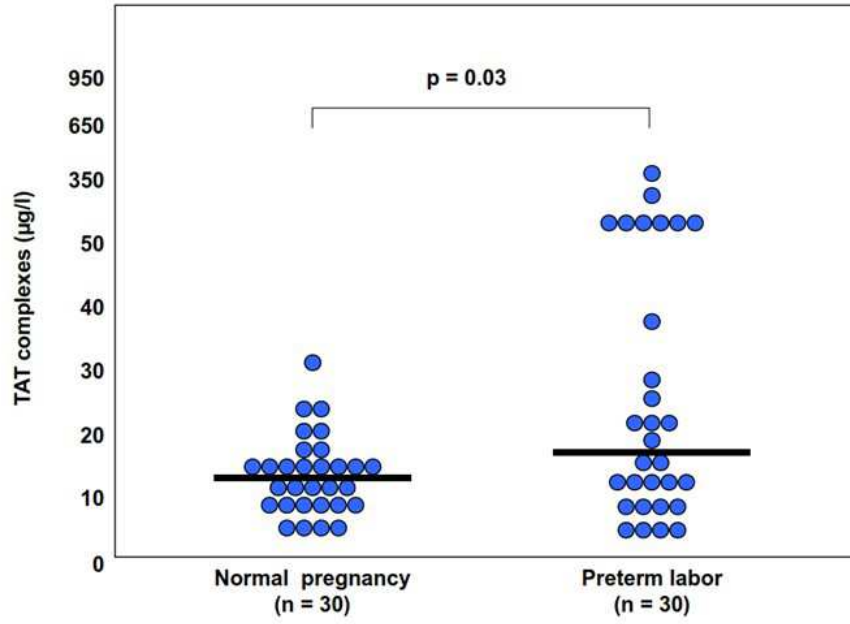


Table 1 (on next page)

Table 1

Concentration and activity in maternal plasma of coagulating and anticoagulating factors and their relation with thrombin generation in the great obstetrical syndromes.

Table 1. Concentration and activity in maternal plasma of coagulating and anticoagulating factors and their relation with thrombin generation in the great obstetrical syndromes.

	TF concentration and/or activity	TFPI concentration and/or activity	TAT III complexes concentration	Protein Z concentration	Thrombin generation	References
Premature rupture of membranes	Activity ↑ Concentration ↑	Concentration ↓	Concentration ↑	Concentration ↓	↑	1-5
Preterm labor	Activity ↑ Concentration =	Activity = Concentration ↓	Concentration ↑	Concentration ↓	↑	1-5
Fetal demise	Activity = Concentration =	Activity = Concentration ↓	Concentration ↑	Concentration ↓	↑	1-5
Preeclampsia	Activity ↑ Concentration ↑	Concentration ↓	Concentration ↑	Concentration ↓	↑	1-5
Intrauterine growth retardation	Concentration ↓	Concentration =	Concentration ↑	Concentration ↓	↑	1-5

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