Classic and Global Hemostasis Testing in Pregnancy and during Pregnancy Complications

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Abstract

Pregnancy is associated with a significant procoagulant shift in the hemostatic system balance as well as other metabolic changes. Pregnancy can thereby provoke manifestation of otherwise dormant disorders of hemostasis (e.g., thrombophilia), or even cause new, pregnancy-specific disorders (e.g., HELLP syndrome). Application and interpretation of laboratory assays of hemostasis in pregnancy is particularly challenging, because normal physiological ranges are no longer applicable, and because the most dangerous and complex changes are not detected by classic routine coagulation/platelet assays. New global assays of coagulation and of platelet-dependent hemostasis appear to be promising in this respect, but are still far from clinical practice and rarely appear in current patient management guidelines. These global assays require a high level of research to identify their relationship to clinically significant outcomes. Here, we review the state-of-the-art knowledge of the molecular changes in the hemostatic system in normal pregnancy and during pregnancy-related complications (preeclampsia, thrombotic microangiopathies, antiphospholipid syndrome, etc.). We also discuss the sensitivity of various classic and innovative assays to these pregnancy-associated changes, and describe current and potential future applications of these assays in meeting specific clinical needs.

Keywords
- normal pregnancy
- pregnancy complication
- classic hemostasis assays
- global hemostasis assays

Pregnancy is associated with major changes in hemostasis, including platelets, coagulation, and fibrinolysis. Procoagulant changes begin early in pregnancy, due to the trophoblast invasion into the endometrium and formation of fetoplacental flow, and then these changes increase with the progress of gestation. These changes predispose women to thromboembolism and other hemostatic disorders during pregnancy and in puerperium, although also supposedly protecting the

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mother from hemorrhage during delivery.[1,2] Some complications during fertilization, pregnancy, and delivery, such as preeclampsia (PE), HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, antiphospholipid syndrome (APS), thrombophilia, or manipulations such as in vitro fertilization (IVF) pregnancy, cesarean section, etc., are particularly risky.[3] For example, the relative risk (standardized incidence ratio) of venous thromboembolism (VTE)[4,5] among pregnant or postpartum women is 4 to 4.5. The first 6 weeks' postpartum are associated with a 22-fold increase of risk, with the peak occurring in the first 3 weeks.[6] Severe obstetric hemorrhage is identified in 1.1% women.[7]

The general description of hemostatic changes in pregnancy and the management of hemostatic disorders is reviewed elsewhere.[1,2,8–14] A specific problem, which is the focus of this article, is the use of contemporary tools of hemostatic laboratory diagnostics in pregnancy. The diversity of hemostatic assays, in combination with a restricted scope of applications, leads to an increasing number of tests for diagnostics. Often the results of some tests contradict one other, making it difficult to achieve a correct diagnosis.[15] For pregnancy, an additional complication is that all normal ranges shift and are not necessarily valid for pregnancy.[16–24] Another critical issue is that traditional routine assays of hemostasis, such as aggregometry and clotting time tests, are far removed from the in vivo conditions[25] and poor in detecting hypercoagulant changes and thrombotic risks, which form a major part of hemostasis complications in pregnancy. Global or integral hemostasis assays are believed to be more sensitive to procoagulant changes, but their clinical application requires solution of numerous problems.[26] This review focuses on the illumination of these issues with a particular attention to the performance of classic and global hemostasis testing in pregnancy and during pregnancy complications.

Hemostasis Assays

Assays can be divided into "functional assays," characterizing a functional state of the system; "individual assays," characterizing the individual system elements; and "marker assays," based on the detection of specific markers of coagulation. Functional assays can be subdivided into subglobal (or "classic") assays, which characterize the work of large system compartments, and "global" assays, tending to better mimic the real clotting in vivo, both including platelet-based and coagulation-based tests. The main features of the assays are shown in ►**Table 1**, and a simplified scheme of hemostasis assays hierarchy is presented in ►**Fig. 1**.

Functional Assays

The first category of assays includes methods that induce hemostatic processes (either coagulation or platelet interaction) in vitro to mimic the in vivo conditions and detect the overall ability of blood to produce a hemostatic plug.

Subglobal (classic) assays include functional tests that have been conventionally used for evaluation of hemostasis for decades.

The main *subglobal clotting assays* are activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) tests. The APTT represents the time to clot formation induced in plasma via the contact pathway, and is sensitive to deficiencies of factors of the intrinsic and common pathways. The APTT is extensively used to monitor unfractionated heparin (UFH) and other anticoagulant agents including direct thrombin inhibitors.[27] The PT uses extrinsic stimulation with tissue factor and is also used as a screening assay to detect deficiencies of one or more coagulation factors (fibrinogen and factors II, V, VII, and X). The international normalized ratio (INR) is the ratio of the patient's PT value divided by the normal value, as determined by the local laboratory, raised to the power of the International Sensitivity Index (ISI) value (usually between 1.0 and 2.0) for the reagent and analytical system used. The PT/INR is used extensively to monitor the anticoagulant effects of warfarin and other vitamin K antagonists and to adjust their dosages. Both APTT and PT do not detect any contribution of circulating active factors, microparticles, etc., as they employ systems that use potent activation and excess lipid.[15] The TT screens for abnormalities in the conversion of fibrinogen to fibrin, and is affected by hypofibrinogenemia, dysfibrinogenemia, and the presence of inhibitors of the fibrinogen-to-fibrin reaction (e.g., heparin, hirudin, dabigatran, fibrin degradation products, and paraproteins).[28]

Subglobal platelet-based tests such as *aggregometry* play a similar role for platelet-dependent hemostasis. For light transmission-based aggregometry (LTA), agonists are added to platelet-rich plasma and an increase in light transmission is recorded as platelets start to aggregate. The method is not well standardized; thus, comparing results between different laboratories is difficult, and LTA is not even close to physiological conditions.[29] In general, LTA was initially designed to assess for potential inherited platelet function disorders, and more recently to monitor treatment response to the common classes of antiplatelet drugs. The recent whole-blood implementations of aggregation comprise Multiplate analyzer (Roche Diagnostics Limited, Switzerland), VerifyNow (Accriva Diagnostics, San Diego, CA), and some others.[30]

Global hemostasis assays represent a new generation of methods,[15,26,31–34] developed to better mimic conditions in vivo[25] and thus be sensitive to a wider range of disturbances in the hemostasis system.

Important *platelet adhesion-based global assays* include the *platelet function analyzer (PFA)* and various videomicroscopy *flow perfusion chambers*. The PFA-100 evaluates the in vitro primary hemostasis by measuring the time required for citrated blood to occlude an aperture in the membrane of a test cartridge, which is coated with various platelet agonists.[35] The PFA-100 is focused on platelet adhesion, with no contribution from blood coagulation being assessed. This assay is believed to be a good indicator of normal platelet-related hemostasis (sensitivity of around 85%), but its specificity for an abnormality in platelet-related function is poor, only 55 to 75%.[36] *Flow chambers* are usually microfluidic devices where the adhesion of platelets to a surface covered with an activator (typically, collagen) under physiological

Table 1 Aspects of standard and global hemostasis assays

Assay	Sample type			Adhesion	Aggregation	Coagulation				Shear	Principle	Standard clinical purpose	Reference
	Plasma	PRP	WB			Init.	Prop	Elast	Lysis				
Platelet assays													
Aggregometry and its modifications	−	+	+	−	+	−	−	−	−	−	Recording the increase of light transmission during agonists' induced platelets aggregation	To monitor treatment response to the common classes of antiplatelet drugs	29,30
Flow cytometry	−	+	+	−	−	−	−	−	−	−	Quantifying the expression of platelet receptors and activation markers using fluorochrome-labeled monoclonal antibodies and agonists		30
PFA and perfusion chambers	−	−	+	+	+	−	−	−	−	+	Monitoring agonist together with shear-induced platelet plug formation, which occludes a capillary		29,30
Measuring of concentrations of coagulation/fibrinolysis markers													
Measuring of concentrations of major proteins of coagulation-fibrinolysis system	+	−	−	−	−	−	−	−	−	−	Measuring of concentrations of factor precursors, inhibitors of coagulation and fibrinolysis markers via clotting assays or ELISA		
Measuring of concentrations of markers of coagulation activation (D-dimer, TAT, F1 + 2)	+	−	−	−	−	(+)	−	−	(+)	−	Measuring of concentrations of markers of coagulation activation and fibrinolysis markers via clotting assays or ELISA	D-dimer—diagnostics of already occurred VTE	230
Sub-global assays (clotting tests)													
APTT	+	−	−	−	−	+	−	−	−	−	Measures the time required for clotting to occur after the intrinsic and common pathway activation	Control of heparin therapy	15,27
PT/INR	+	−	−	−	−	+	−	−	−	−	Measures the time required for clotting to occur after the addition of a source of tissue factor (extrinsic pathway activation).	Control of anti-vitamin K prophylaxis	15,27

(*Continued*)

Table 1 (*Continued*)

Assay	Sample type			Adhesion	Aggregation	Coagulation				Shear	Principle	Standard clinical purpose	Reference
	Plasma	PRP	WB			Init.	Prop	Elast	Lysis				
TT	+	−	−	−	−	+	−	−	−	−	Screens for abnormalities in the conversion of fibrinogen to fibrin (hypofibrinogenemia, dysfibrinogenemia, and the presence of inhibitors of the fibrinogen-to-fibrin reaction (heparin, hirudin, fibrin degradation products, and paraproteins)	Is used primarily to evaluate plasma specimens with prolonged activated partial thromboplastin time (APTT) values and, to a lesser extent, prolonged prothrombin time (PT)	28
Global assays													
TEG/ROTEM	−	(+)	+	−	−	−	+	+	+	−	Monitoring clot formation in whole blood using agonists	Control of bleeding and transfusion in surgery	29,30,231
TGT	+	+	−	−	−	+	+	−	−	−	Thrombin concentration as a function of time is obtained from the cleavage of chromogenic or fluorogenic substrate		26,30
OHP	+	−	−	−	−	+	(+)	−	+	−	Based on repeated spectrophotometric registration of the fibrin-aggregation curve in platelet-poor plasma containing small amounts of exogenous thrombin, tissue-type plasminogen activator, and calcium		30
Thrombodynamics	+	+	(+)	−	−	+	+	−	−	−	Coagulation is detected in a cuvette by time-lapse image capture of light scattering from the fibrin network		30

Abbreviations: APTT, activated partial thromboplastin time; F1 + 2, prothrombin fragment 1 + 2; OHP, overall hemostasis potential; PFA, platelet function analyzer; PT/INR, prothrombin time/international normalized ratio; TAT, thrombin–antithrombin III complex; TEG/ROTEM, thromboelastography/rotational thromboelastometry; TGT, thrombin generation test; TT, thrombin time.

Notes: This table represents assays ability to measure adhesion, aggregation, coagulation in terms of initiation (Init.), propagation (Prop.), clot elasticity (Elast), and fibrinolysis (Lysis) (mostly adapted from Tynngard et al Thromb J 2015;13:8). The table shows the type of sample (plasma, platelet-rich plasma [PRP] or whole blood [WB]) that can be assessed in each assay. The table also shows if the measurement can include the contribution by shear components. Plus (+) means yes and minus (−) means no; signs within parentheses means possible in theory, but not commonly used.

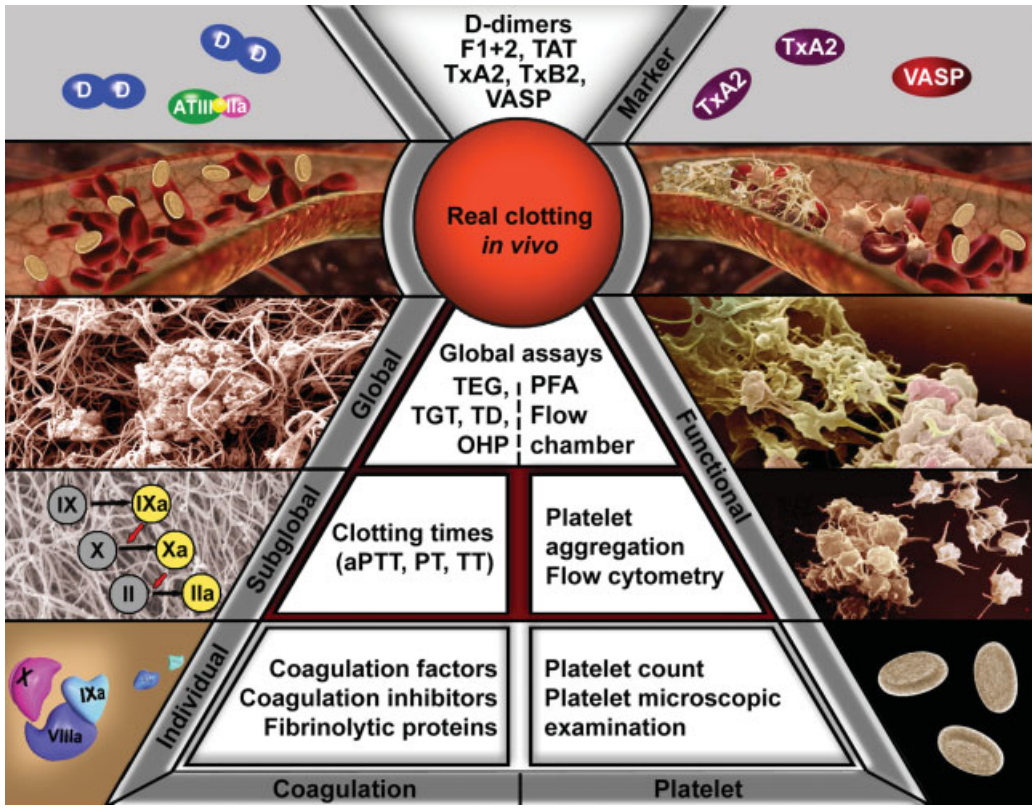

Fig.1 Scheme of hemostasis assays classification based on hemostasis coverage: from individual (differential assays) to subglobal (standard assays), global (new assays), and after the event (markers of thrombosis).

flow conditions is monitored by microscopy observation. The use of flow-based assays for assessment of hemostasis has been recently reviewed by the International Society on Thrombosis and Hemostasis (ISTH) Scientific Subcommittee (SSC) Biorheology.[30] Some of the platelet-based flow chamber assays, such as T-TAS, may include formation of fibrin as well.[37]

Coagulation-based global assays are numerous and differ in their design. One way to characterize clot formation is by rheometry, which has additional advantage of being easily applied in whole blood. *Thrombelastography (TEG)/thromboelastometry* (TEM) is the most ancient global assay of hemostasis, where clot formation and platelet aggregation are evaluated simultaneously using forced oscillation rheometry, and probably the only one that has now become more widely used in clinical practice despite many ongoing concerns.[32] *Thrombin generation*, invented in its present form by the team of Coenraad Hemker of Maastricht University,[38] uses a thrombin-sensitive fluorogenic substrate to detect thrombin concentration as a function of time. The curve usually has a characteristic bell shape. Such parameters as endogenous thrombin potential (ETP, area under the thrombin generation curve) are among the most widely used, and their correlation with clinical phenotype is well established, although standardization issues still exist. There are presently numerous modifications of thrombin generation, including several commercially available versions. It appears that thrombin generation is sensitive to various hypercoagulation factors depending on the design, including sensitivity to factors II and V, fibrinogen (Fg), antithrombin (AT) at high tissue factor (TF) (13.6 pM); to factor XII, Fg, AT, free tissue factor pathway inhibitor (TFPI) at low TF (1 pM),[39] as well as to factors VIII and IX[40]; to protein C pathway defects upon addition of thrombomodulin (TM) or protein C activator[26]; to circulating TF when performed without activators; and to lipids when performed without externally added lipids.[26] Fibrinolysis and use of whole blood are currently beyond the available versions of this method, although some preliminary data on thrombin generation in whole blood has appeared.[41] The *overall hemostasis potential (OHP)* is based on repeated spectrophotometric registration of the fibrin-aggregation curve in platelet-poor plasma containing small amounts of exogenous thrombin, tissue-type plasminogen activator, and calcium. The overall coagulation potential and overall fibrinolytic potential are supplementary parameters of OHP, with studies reported in several hyper- and hypocoagulable states and during anticoagulant treatment.[30,42] Finally, *thrombodynamics* is based on the idea of monitoring spatial fibrin formation initiated by immobilized TF in plasma by video-microscopy, so that clot is initially formed on the activator and then propagates into plasma. The idea behind this is to take into account spatial heterogeneity of blood coagulation; in other words, the fact that clotting initiation and propagation occurs in spatially separated regions.[25] In agreement with the wound clotting in vivo, TF is located on the surface, and clot propagates because of coagulation factor activation and diffusion.[43–47] Importantly, separation of the activation and propagation phases[48] makes the assay particularly sensitive

to the presence of coagulation activators in plasma such as circulating TF or factor XIa. Spatial clot formation rate indicates overall procoagulant potential, while formation of activator-independent spontaneous clotting centers may indicate presence of microparticles and long-lived coagulation factors.[26]

Individual Assays

The second category of methods includes assays that determine specific parameters of hemostatic systems. A typical example is the wide palette of approaches aimed at the determination of individual *protein concentrations* using either clotting-based or enzyme-linked immune-labeling assays. For platelet-dependent hemostasis, the same function is performed by *flow cytometry*, which can identify deficiency or defects in almost all essential platelet glycoproteins or activation responses.[29] Finally, there are numerous other specific assays (e.g., luminescence-based determination of dense granule release, determination of von Willebrand factor [VWF] multimers and a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 [ADAMTS13] activity, etc.), but these are beyond the scope of this article.

In general, these differential methods can identify specific problems (and are thus indispensable in the investigation of isolated inherited hemostasis disorders), but do not provide a general picture of hemostasis and are simply too limited: hemostasis is too large a system, and there are many components that cannot be measured as a part of routine real practice.

Marker Assays

The final class of coagulation assays monitors *the markers of thrombosis that has already occurred*: D-dimers, thrombin–antithrombin (TAT) complexes, intermediate forms of activated proteins. These assays help diagnose thromboses and thromboembolisms and can predict future thrombotic complications with certain conditions.[49] These assays are usually based on specific antibodies.

Hemostatic Changes during Normal Pregnancy

Molecular Changes in the Hemostatic System

The overall balance of the coagulation network in pregnancy is shifted to a procoagulant state. Concentrations of *coagulation factors* (see ►**Table 2**) VII, VIII, IX, and XII are increased up until 5 to 8 weeks postpartum.[50–53] The concentration of VWF antigen rises to become up to five times higher than the prepregnancy state.[51,53–56] Factors II, X, and V remain within nonpregnant reference intervals.[50,54] Information about factor XI is controversial.[50,52,53,57,58] The fibrin-stabilizing factor XIII shows a progressive decline.[59] Plasma fibrinogen levels steadily increase during pregnancy up to twice that of the nonpregnant level.[50,52,54,58,60,61] Importantly, circulating levels of active factors XII and VII are also increased during normal pregnancy.[62] The plasma hypercoagulation in normal pregnancy is confirmed by the presence of a plurality of fibrin deposits and zones of "fibrinoid necrosis" with fibrin deposits (up to 7% of the chorionic villous area) in histological samples of placenta. These data are confirmed by immunohistochemistry.[63–67]

Pregnancy is generally also associated with a decrease in *coagulation inhibitors* (see ►**Table 2**). The AT level remains reasonably stable during pregnancy, delivery, and the postpartum period, at levels slightly lower than the nonpregnant reference interval.[9,50,60] Heparin cofactor II and TFPI levels are higher during pregnancy than in nonpregnant women.[9,68–70] Levels of protein C (PC) and total protein S (PS) appear to remain constant during normal pregnancy,[50,52,53,60,71,72] though this is disputed.[73] PS activity and free PS gradually decrease during pregnancy.[50,53,60,71]

The overall effect of pregnancy on *fibrinolysis* is unclear (see ►**Table 2**). Plasminogen levels, tissue plasminogen activator (t-PA), and urokinase-type plasminogen activator (u-PA) antigen increase throughout the pregnancy,[9,52,72,74–78] whereas t-PA activity decreases remarkably.[74,78] There occurs a rise in levels of both plasminogen inhibitors (PAI-1, which increases up to four times the nonpregnant values,[52,60,75–77,79] and PAI-2, which is placenta-derived, increases five times until delivery, compared with the I trimester values).[60,75,77,79] The fast plasmin inhibitor α_2-antiplasmin is unchanged during pregnancy.[58] Thrombin-activatable fibrinolysis inhibitor (TAFI) level during pregnancy is reported to remain unchanged[80] or increases.[81,82] There is no evidence of increase in fibrinolytic activity associated with pregnancy estimated with the CLI30 parameter in TEG or ROTEM assays.[83] The reason for this may be because of these assays' insensitivity to hypofibrinolysis (caused by increased concentration of plasmin activator inhibitors PAI-1 and PAI-2); for example, the normal range of the ROTEM CLI30 is 94 to 100% (median 98%),[84] and decreased fibrinolysis will show higher than normal values (>98%), making it impossible to distinguish hypofibrinolysis from normal lysis. The modified tissue factor-induced ROTEM with addition of tPA also revealed no difference in fibrinolysis profiles between pregnant and nonpregnant groups.[85] This assay has another drawback: the concentration of TPA in the sample exceeds the highest possible concentration of fibrinolysis inhibitors by several times, which makes system insensitive to any impairments in fibrinolysis.

Changes in *platelet function* during normal pregnancy (see ►**Table 3**) are much less studied and poorly understood compared with those in blood coagulation. It seems established that platelet count decreases by approximately 10%.[60] However, the significance of such changes is unclear, as much greater changes are usually required to cause bleeding or thrombosis. Clinical data indicate no bleeding due to gestational thrombocytopenia.[86]

There seems to be a substantial number of studies reporting slight to moderate increase of platelet reactivity and preactivation in the third trimester of healthy pregnancy based on the aggregometry and flow cytometry data.[87–95] This hyperaggregability could be related to the decreased basal cAMP levels and elevated calcium mobilization.[92,95] On the other hand, several studies mostly employing flow

Table 2 Major changes in coagulation factors, coagulation inhibitors, and fibrinolysis parameters concentrations during pregnancy in relation to the nonpregnant state

	Overall change during pregnancy, compared with nonpregnant state	Comparative values, % of nonpregnant state or 5–8 wk postpartum	References
Coagulation factors			
II	=	–	50, 54
V	=	–	50, 54
VII	↑	150–180	50, 52
VIII	↑	200–300	50–52
IX	↑	150–200	50, 52
X	=	–	50, 54
XI	=/↓	60–100	50, 52, 53, 57
XII	↑	120–130	50, 53
VWF	↑	200–500	51, 53–56
XIII	↓	70	59
Fg	↑	120–200	50, 52, 54, 58, 60, 61
Inhibitors of coagulation			
Antithrombin III	=	–	9, 50, 60
Heparin cofactor II	↑	120–130	9, 68, 69
TFPI	↑	140	69, 70
Protein C	=	–	50, 53, 60, 71, 72
APC ratio	↓	80	73
Total protein S	=	–	50, 52, 60, 72
Free protein S	↓	50–80	50, 53, 60, 71
Protein S activity	↓	60	50
Thrombomodulin	↑	140–150	76, 81
Fibrinolysis			
Plasminogen	↑	130–170	9, 78
t-Pa antigen	↑	160–190	52, 72, 74–77
t-Pa activity	↓	4–10	74, 78
u-Pa antigen	↑	120[a]	77
PAI-1	↑	170–700	52, 60, 75–77, 79
PAI-2	↑	3,000–15,000	60, 75, 77, 79
α_2-antiplasmin	=	–	58
TAFI	↑/=	100–130	80–82

Abbreviations: APC ratio, activated protein C ratio; Fg, fibrinogen; PAI-1, plasminogen activator inhibitor 1; PAI-2, plasminogen activator inhibitor 2; TAFI, thrombin-activated fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; t-Pa, tissue plasminogen activator; u-Pa, urokinase plasminogen activator; VWF, von Willebrand factor.
List of symbols: "↑," higher than values for nonpregnant state; "↓," lower than values for nonpregnant state; "↑/↓," contradictory results in different papers; "=," no difference between nonpregnant state values and values during pregnancy.
[a]No data about nonpregnant state, the change is compared with first trimester.

cytometry reported that platelet activation in pregnancy remains normal or even decreases.[88,96–98] Basal cAMP level was also reported to be unchanged.[99]

Of particular interest with regard to this review is a recent study[88] that employed assays of platelet adhesion in flow perfusion chambers to characterize integral platelet function during pregnancy. The authors demonstrated that platelet thrombus formation is impaired in healthy pregnancy, despite increased aggregation and unchanged flow cytometry markers measured in the same study. Their previous study on intravital thrombus formation in mice[100] revealed that estradiol treatment has a profound effect on the expression of

Table 3 Major changes in platelet parameters and microparticles concentration during pregnancy in relation to the nonpregnant state

	Overall change during pregnancy, compared with nonpregnant state	Comparative values, % of nonpregnant state or 5–8 wk postpartum	References
Platelets			
Platelet number	↓	90	60
Aggregation	↑	110–140	87, 91, 94, 95
Activation markers	↑/↓	80–150	88, 96–98
Adhesion	↓	90	88
Microparticles			
Annexin-V microparticles	↑	250	104
Platelet-derived microparticles	↑	185	104
P-Selectin +, activated platelet-derived microparticles	↑	150	104
Endothelial-derived microparticles	↑	190	104
Leukocyte-derived microparticles	↑	230	104
Erythrocyte-derived microparticles	↑	140	103
Tissue factor-bearing microparticles	↑	530	104
Placenta-derived microparticles	↑	270	103
Phospholipid clotting time	↓	55	104

List of symbols: "↑," higher than values for nonpregnant state; "↓," lower than values for nonpregnant state; "↑/↓," contradictory results in different papers; "=," no difference between nonpregnant state values and values during pregnancy.

many platelet proteins, resulting in decreased thrombosis risk.

Although additional research and larger patient cohorts are required in order for these very recent data to become more established and to potentially influence clinical practice, they illustrate a critical difference between the three strategies of clinical laboratory evaluation of platelet reactivity: hyperaggregability in the aggregation assays and normal activation markers in flow cytometry are in sharp contrast with the decreased ability to form thrombi in flow. This illustrates the role and the importance of the integral assessment of platelet function.

Microparticles (MPs) of all types are increased throughout pregnancy (see ►**Table 3**), possibly due to the increased blood flow through the placental bed.[101,102] MPs gradually increase according to the gestational week, with the highest values reached in the third trimester.[103,104] Phospholipid clotting time is significantly shorter in the three trimesters of pregnancy as compared with controls.[104]

Assays of Hemostasis during Normal Pregnancy

Classic clotting assays, such as APTT, TT, PT, and INR, do not reveal any changes throughout the pregnancy or puerperium and remain stable at nonpregnant values[11,50,61,72,94] (see ►**Table 4**).

Global hemostatic assays, such as Thromboelastography (TEG) and rotational thromboelastometry assay (ROTEM) reveal increasing hypercoagulation during normal pregnancy progress.[18,20] At all times during pregnancy, and regardless of the test used (EXTEM, INTEM, and FIBTEM), no significant change in CT is observed. However, by contrast, MCF gradually increases up to 1.5 times of nonpregnant values from the first trimester of pregnancy onwards. This increase is maximal and significant during the second trimester of pregnancy and persists into the third trimester. The early amplitude variables CA5 and CA15 for the INTEM, EXTEM, and FIBTEM tests are significantly higher in women in the second and third trimesters of pregnancy compared with nonpregnant women.[83,105]

There are reports on the increase in ETP and peak height from I to III trimester using the *Thrombin Generation Test* (see ►**Table 4**), while lag time and time to peak remain unchanged,[70] although some authors reveal no change in any test parameters throughout the pregnancy or no increase in early pregnancy.[24,106] There are data suggesting dependence of thrombin generation parameters on concentrations of factor VIII, and TFPI as those components believed to be responsible for the procoagulant shift seen in pregnancy.[107,108]

The lag time in thrombodynamics does not change during uncomplicated pregnancy.[16,109] Stationary clot growth rate (Vst) has a shift toward hypercoagulation during first trimester and stays stable until the delivery[16] or has a slight gradual increase from one trimester to another.[109] Clot density (D) shows a gradual increase with the gestation progress[16] (see ►**Table 4**).

Markers of coagulation activation are increased during pregnancy (see ►**Table 4**). Prothrombin fragment 1 + 2

Table 4 Major changes in hemostasis assay parameters and coagulation activation marker concentrations during pregnancy in relation to the nonpregnant state

	Overall change during pregnancy, compared with nonpregnant state	Values, compared with nonpregnant state or 5–8 wk postpartum (%)	References
Coagulation tests parameters			
APTT	=	–	11, 50, 61, 72, 94
TT	=	–	11, 50, 61, 72, 94
PT	=	–	11, 50, 61, 72, 94
INR	=	–	11, 50, 61, 72, 94
TEG/ROTEM parameters			
R/CT	↓/=	60–100	18, 20, 83, 105
K/CFT	↓	50–90	18, 20, 83, 105
MA/MCF	↑	110–150	18, 20, 83, 105
CLI30	=	–	18, 20, 83, 105
Thrombin generation test parameters			
ETP	↑/=	150	24, 70, 106
Peak height	↑/=	120	24, 70, 106
Lag time	=	–	24, 70, 106
Time to peak	=	–	24, 70, 106
Thrombodynamics parameters			
Tlag	=	–	16
Vi	↑/=	100–110	16, 109
Vst	↑/=	100–140	16, 109
CS	↑/=	100–120	16, 109
D	↑	100–130	16, 109
Markers of coagulation activation			
F1 + 2	↑	370	78
TAT	↑	430	60
Fibrinopeptide A	↑	130	111
D-dimer	↑	210–850	19, 22, 61, 112–116

Abbreviations: APTT, activated partial thromboplastin time; CLI30, clot lysis index after 30 minutes; CS, clot size; D, clot density; ETP, endogenous thrombin potential; F1 + 2, prothrombin fragment 1 + 2; INR, international normalized ratio; K/CFT, clot formation time; MA/MCF, maximum clot firmness; PT, prothrombin time; R/CT, clotting time; TAT, thrombin–antithrombin III complex; TEG/ROTEM, thromboelastography/rotational thromboelastometry; Tlag, lag time; TT, thrombin time; Vi, initial clot growth rate; Vst, stationary clot growth rate.
List of symbols: "↑," higher than values for nonpregnant state;" ↓," lower than values for nonpregnant state; "↑/ ↓," contradictory results in different papers, " = ," no difference between nonpregnant state values and values during pregnancy.

(F1 + 2), TAT, and fibrinopeptide A show a gradual increase.[60,79,110,111] Levels of D-dimer gradually increase with gestation progress and normalize within 8 weeks postpartum.[19,22,61,112–116] TM levels increase throughout pregnancy; some authors state normalization in postpartum period[76] and some state that TM level remains high in postpartum.[81]

Assays of Hemostasis in Complicated Pregnancy

Preeclampsia

Preeclampsia and eclampsia are the leading obstetric causes of direct maternal deaths.[117] PE is a pregnancy complication that is typically characterized by new-onset hypertension and proteinuria after 20 weeks of gestation and affects both mother and fetus. PE is defined by high blood pressure on two occasions (≥140 mm Hg systolic or ≥90 mm Hg diastolic) combined with proteinuria (≥0.3 g protein in a 24-hour urine specimen) during the second half of pregnancy.[118–121] Accurate incidence figures are difficult to obtain, and the incidence varies between countries, but it is believed that worldwide, 3 to 5% of pregnant women are affected.[122] Eclampsia is a severe complication of PE during pregnancy or postpartum in a woman with signs or symptoms of PE. The incidence of eclampsia in women with PE is 2.6%.[123] PE is the leading cause (23.6%) of perinatal death in economically poor countries.[124]

In economically rich countries, PE is less lethal in an absolute sense, although the condition is responsible for around 13% of maternal deaths.[125]

VTE is associated with severe PE, as PE significantly increases the possibility of thromboembolism.[3,126] VTE risk is increased up to 20 cases per 10,000 pregnancies and 10 times higher than VTE risk for nonpregnant women. Incidence is even higher in women with eclampsia: 106 per 10,000 eclamptic pregnancies for the postpartum period.[3]

The pathogenesis of PE is not well understood, and the only treatment proven to be effective is delivery. It is believed, however, that a major role is played by the activation of the hemostatic system in the placenta, possibly as a result of its abnormal development. Shift in hemostatic balance likely leads to formation of microthrombi and dysfunction of some organs. Ischemia that occurs due to destruction of the endothelium and fibrin deposits leads to the formation of an infarction zone in the placenta.[127] Infarction was seen in 80.2% (95% confidence interval [CI]: 72.9–87.5) of severe PE, 61.0% (95% CI: 46.1–75.9) of mild PE, and in 20.4% (95% CI: 14.1–26.7) of non-PE placentas. Impaired renal function in PE women is accompanied by endothelial and mesenchymal tissue damage as well as the emergence of significant deposits of fibroid necrosis with fibrin deposits in the subendothelial space of renal tissue.[128]

Placenta-derived MPs, syncytiotrophoblast MPs (STBM), play an important role in the pathogenesis of PE and other abnormal pregnancies. Increased levels of STBM have been reported in PE when compared with gestation-matched healthy individuals.[129,130] MPs are very likely involved in the hypercoagulable and proinflammatory intravascular reactions during PE.[131] Mice injected with phosphatidylserine/phosphatidylcholine microvesicles showed a significant elevation in systolic blood pressure, a significant increase in TAT level, a significant decrease in platelet count, a decrease in AT, an increase in proteinuria, and a significant reduction in fetal weigh and placental weight, compared with controls.[132] PE-like symptoms were significantly alleviated after the phosphatidylserine/phosphatidylcholine microvesicles–injected mice were treated with annexin V, hirudin, or heparin.[133] Furthermore, fibrin deposition in the placentas in the anticoagulant-treated mice was remarkably improved, compared with that in the mice injected with phosphatidylserine/phosphatidylcholine alone.

Screening and early identification of women at risk of PE could enable appropriate application of antenatal care, management, and treatment.[134]

The concentration of coagulation factors is changed in both directions in PE women, but levels essentially remain within the normal reference range.[57,135–137] Clotting times (APTT, PT, and TT) and fibrinogen level also remain within the normal reference range.[128,135,137] The concentration of the coagulation inhibitors (PC, PS, and AT) is slightly reduced or unchanged in PE compared with normotensive pregnancy[126,136–138]; on the contrary, TFPI level is elevated.[136,139,140] The soluble thrombomodulin activity is increased.[140] The data are conflicting about the soluble tissue factor (sTF).[139,140] Platelet activity is increased: the percentage of CD62P+ platelets, CD62P+ platelet MPs, and platelet-monocyte aggregates are significantly higher in women with PE than in pregnant controls,[141,142] but the platelet count is slightly decreased especially in severe PE.[135] The fibrinolytic system in PE is characterized by elevated PAI-1 and depressed PAI-2 levels.[126,136,140] Women who developed PE had significantly higher ETP than normotensive pregnant controls.[136,141,143–145] Lag time of thrombin generation is shortened in PE.[143,145] Patients with PE had a higher plasma TAT complex, F1 + 2, and D-dimer concentration than normal pregnant women,[126,128,136,140,144,146,147] but other work has shown that the TAT level in PE was not significantly higher compared with normotensive pregnancy.[148] TEG does not demonstrate any changes in PE compared with normotensive pregnancy,[135,137,149–151] except for a maximal amplitude decrease with the platelet count.[135] Clot lysis time in TEG is shortened in PE.[149,150] Only in women with severe PE, "r" and "k" are slightly increased and "α" is decreased.[151] Preliminary data obtained with thrombodynamics assay in Kazan State Medical University (Kazan, Russia) show that women with PE that developed in the third trimester ($n = 20$) had clot growth rate increased by 20% compared with the group of healthy pregnant women ($n = 94$) at the similar gestational age. Half of the patients showed spontaneous clotting in the thrombodynamics assay. Another study (Municipal Hospital # 11, Chelyabinsk, Russia) with thrombodynamics showed that women with chronic placental hypoperfusion in the third trimester ($n = 27$) had an increased rate of spontaneous clotting (59 vs. 35%, $p = 0.04$) compared with the group of healthy women ($n = 57$) and decreased time of spontaneous clotting (13 vs. 18 minutes, $p = 0.007$).

From a clinical point of view, although PE is believed to be a disorder caused by the hemostatic system malfunctioning in placenta, its detection, identification, and treatment currently do not rely on assays of hemostasis. The parameters of these assays do not change reliably under such disease states, and these assays are not a part of any international guidelines for treatment or diagnosis of PE. Still, at least one important potential utility of the hemostasis assays is the above-stated lack of significant changes; thus, as soon as hemostasis assay parameters begin to change, it might be an indication of transition of PE to the HELLP syndrome, which is the subject of the following section.

Thrombotic Microangiopathies

Pregnancy is known to be a major precipitating event for the development of various thrombotic microangiopathies (TMAs)[14]: their frequency in pregnancy is essentially increased, they may come in pregnancy-specific forms, and can be recurrent over consecutive pregnancies. TMAs are pathological conditions usually defined as formation of disseminated microthrombi in the microvascular circulation. They share several similarities with disseminated intravascular coagulation (DIC) including consumptive thrombocytopenia and anemia. However, they are differentiated from "true severe DIC" based on their slower and more compensated and less disseminated character. In TMAs, there is no coagulopathy at the early stages, and no multiple organ dysfunction.[152]

Still, TMAs dangerously predispose the patient to DIC and other complications, and may progress to DIC in 20 to 40% of cases. The specific molecular mechanisms leading to development of TMAs in pregnancy are poorly understood, but are believed to be a combination of endothelial dysfunction and procoagulant changes in blood plasma. Depending on the main organ/system affected, three main categories of TMAs are recognized:

1. Thrombotic thrombocytopenic purpura (TTP), a classic condition characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, and dysfunction of renal and central nervous systems. In pregnancy, TTP is fivefold more frequent than in the nonpregnant population, and usually occurs in the second trimester.
2. Pregnancy-associated HUS is believed to be predominantly atypical HUS (aHUS), that is, complement-mediated.[153] It differs from TTP by a more severe renal dysfunction and a milder neurological dysfunction; usually occurs postpartum.
3. The HELLP syndrome, a severe variant of PE, characterized by liver dysfunction in addition to anemia and thrombocytopenia. This condition is specific to pregnancy and supposedly has a root cause in poor placenta perfusion. It can interact with other disorders and is relatively frequent (up to 0.8% of pregnancies). It is usually observed in the third trimester, although it may occur postpartum.

From the hemostasis laboratory diagnostics point of view, all three conditions share several common TMA markers: decreased platelet count, schistocytes, increased fibrin degradation products, but normal APTT and PT. They also share nonhemostatic markers such as anemia, reticulocytosis, increased bilirubin and lactate dehydrogenase, decreased haptoglobin, and reticulocytosis. The critical issue is differentiation between these three conditions, because the treatment strategies for HELLP, TTP, and aHUS essentially differ.[154,155] Differentiation is presently done using a combination of hemostatic markers like ADAMTS 13 activity[154]; nonlaboratory indicators, such as specific types of pain and hypertension in HELLP[14]; or nonhemostatic markers such as a basic metabolic panel.[156] In severe, DIC-threatening cases, when coagulopathy begins to develop and organ dysfunction spreads, the difference between the three TMAs becomes less pronounced.[14] APS can also be associated with TMAs.[157]

There is little information about use of global assays in the management of TMAs in pregnancy. A single study on the use of TEG in HELLP syndrome reported two cases, where it was able to differentiate between the two mechanisms of bleeding and improve treatment.[158]

Antiphospholipid Syndrome

APS is a systemic autoimmune disease characterized by recurrent arterial or venous thrombosis and/or recurrent pregnancy morbidities in the presence of persistent positive antiphospholipid antibodies (aPL), which include anticardiolipin antibodies (aCL), anti-β2 glycoprotein I (anti-β2GPI), and lupus anticoagulant (LA).[159]

Pregnancy-associated complications of APS affect both the mother and the fetus. These include fetal death (which can occur early or late), intrauterine growth retardation, premature delivery, and dysmaturity. The rate of adverse pregnancy outcomes in women with APS depends on the severity of APS, the history of prior obstetric complications or thrombotic events, and the treatment strategy.[160–162] Preterm delivery is reported in around 20% of APS patients.[8] Placental infarction is a feature of fetal loss in some cases of APS, suggesting a thrombotic pathogenesis. One postulated mechanism is that aPL displaces annexin V (a potent anticoagulant protein) from trophoblasts with resulting increased exposure of anionic phospholipids and acceleration of thrombin generation.[8,163] In addition, the mother can suffer from venous and/or arterial thrombosis. The rates of deep vein thrombosis (DVT;1.46%; range, 1.15–1.82%), pulmonary embolism (0.43%; range, 0.26–0.66%), superficial vein thrombosis (0.44%; range, 0.28–0.68%), and cerebrovascular events (0.32%; range, 0.18–0.53%) are significantly higher in aPL-positive women than in the other groups despite low-dose aspirin primary prophylaxis.[164] The risk of thrombosis in APS can be estimated with the Global Anti-Phospholipid syndrome Score (GAPSS) guideline.[165] The strategy of anticoagulant treatment is described elsewhere.[166]

The classification criteria for APS in 2006 requires the presence of one positive clinical criterion whether manifested by thrombosis or pregnancy loss plus one positive laboratory criterion (positive aPL, this can be any antibody of the three antibodies mentioned above) on two different occasions separated by 12 weeks.[167] However, these criteria cannot be fully considered as diagnostic, despite commonly used as such. Recommendations for improving the laboratory diagnostics of APS can be obtained elsewhere.[168]

aPL is identified using a large number of laboratory procedures based on one of two distinct test processes, namely, solid-phase assays and liquid-phase assays.[168] No systematic data has been obtained regarding global hemostasis assays in obstetric APS patients. As with other pregnancy complications, there are case studies, for example, on decision making when dealing with a case of cesarean delivery in complicated APS when TEG was employed.[169]

Thrombophilia

Inherited thrombophilia is defined as a genetic predisposition to VTE, usually a genetic deletion or alteration of a functional protein involved in coagulation. The major heritable forms of thrombophilia include deficiencies of AT, PC, and PS; abnormalities of procoagulant factors, particularly factor V Leiden (FVL); and the prothrombin G20210A gene polymorphisms. Pregnancy greatly increases the risk of VTE in thrombophilia (up to 34.4% for homozygous FVL); other complications like pregnancy loss are also widespread in affected patients.[170]

Because hypercoagulability with inherited thrombophilia has been well established, genetic screening of pregnant women with a personal history of VTE has been generally well accepted in practice, with the purpose of providing thromboembolic prophylaxis if needed. This practice is supported by the most recent guidelines, and its acceptance is

confirmed in the authors' survey findings of physicians' practices. A controversy existed in the recent past with regard to the utility of screening for inherited thrombophilia in women with a history of adverse pregnancy outcome or loss.[171–176]

The heparin treatment strategy recommendations in patients with thrombophilia can be obtained elsewhere.[171,175]

With regard to nongenetic types of assays, levels of D-dimer were significantly higher in women with FVL than in those without the mutation, both during pregnancy and puerperium.[106,177] However, other thrombosis markers, such as F1 + 2 and TAT, as well as fibrinogen levels were not increased.[106,177,178] The global hemostasis assays show ambiguous behavior. Both peak thrombin and ETP were increased over the course of pregnancy compared with the nonpregnant state (8 weeks postpartum) in women with mild thrombophilia (women with heterozygosity for FVL or Prothrombin 20120A mutation and/or a positive history for VTE and/or a positive family history for VTE) as well as those with no thrombophilia.[179] On the contrary, other authors demonstrated that the ETP remained unchanged in both women with and without FVL at all time points (12th, 22nd, and 34th gestational weeks as well as 3 months after delivery).[106] Parameters of TEG "r," "k," and TMA increased while α-angle decreased in patients with inherited thrombophilia as compared with controls.[149] There was no correlation observed in studies[180] between TEG parameters and other thrombophilia-related defects (PC, PS, FVL mutation, prothrombin G20210A mutation, MTHFR C677T mutation, and lupus anticoagulant). Levels of OHP, clotting time, and clot lysis time in women who had previously experienced DVT in connection with pregnancy and heterozygotes FVL mutation were increased compared with the healthy individuals.[178] Another group examined whether pregnant patients with established thrombophilic disorders demonstrated a decreased response to TM, favoring a prothrombotic tendency.[181] The thrombophilia (FVL or prothrombin 20210A gene mutations as well as PS, PC, or AT deficiencies; hyperhomocysteinemia; aPL; or lupus anticoagulant) group was noted to have significantly lower TACT ratios (assay measures the effect of thrombomodulin on the APTT) compared with the outcome of normal pregnancy patients (mean 1.88 ± 0.32 vs. 2.14 ± 0.53; $p < 0.02$). To summarize, there is some information about the sensitivity of different functional assays and markers to thrombophilia in pregnancy, but no current guidelines support decision making based on nongenetic assays.

Gestational Diabetes

Diabetes reflects a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Gestational diabetes mellitus (GDM) is defined by the World Health Organization as having "any degree of glucose intolerance with onset or first recognition during pregnancy."[182] GDM is usually detected in the second half of pregnancy, when pancreatic function is not sufficient to overcome the diabetogenic environment of pregnancy.[183] The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues.[184] The prevalence of GDM in pregnant women varies substantially, ranging from 1.7 to 11.6% in advanced economies.[185] In Asian countries, the prevalence varies according to the screening strategy and diagnostic criteria and ranges from 1 to 20%, with an increasing trend being evidenced in recent years.[186]

Adverse pregnancy outcomes associated with diabetes include hydramnion, diabetic fetopathy, and fetoplacental insufficiency (all of which have negative influence on fetus) and hypertensive complications such as gestational arterial hypertension and PE (which have a negative influence on the mother).[187,188] The incidence rate of VTE in pregnancy, complicated with diabetes, is reported to reach 2.3% (OR: 4.1, range: 2.0–8.9), which is 23-fold higher than in normal pregnancy.[189]

The screening strategy for GDM is reported in international guidelines for diabetes in detail.[183,184] No special guidelines were found for heparin treatment in GDM patients.

There are no significant changes in platelet count or platelet adhesion in the gestational diabetic group compared with normal pregnancies; however, mean platelet volume is higher in the gestational diabetic group.[190,191] Platelet aggregation is also increased in the group of gestational diabetics compared with the group of normal pregnancies.[192] The fibrinogen level is significantly higher in GDM women during pregnancy compared with normal pregancy.[193] Higher levels of TAT, t-PA, and D-dimer as well as lower levels of PC have also been reported in gestational diabetes, in comparison with normal pregnancy.[68] Conflicting data are provided about total and free PS levels in pregnancy complicated with GDM.[68,193] Akinci et al[194] found elevated PAI-1 levels in pregnant women with gestational diabetes compared with normal pregnancy, whereas Winzer et al[195] and Bellart et al[68] failed to find such a difference. No difference in PAI-2 is revealed.[68] During pregnancy, women with GDM have higher t-PA levels than normal women.[68] TM levels in pregnancy do not differ between the normal and GDM groups. Plasma TAFI antigen levels are significantly higher in pregnant women with GDM when compared with pregnant controls.[196] APTT, PT, and TT assays reveal no difference between normal pregnancy and GDM pregnancy group.[193] The TEG parameters in the GDM patients show hypercoagulable state changes compared with the control group, but the differences are not significant.[197] We are not aware of data obtained for the thrombin generation test in GDM pregnancy. Although the pathogenesis of the disease is related to hemostasis, no guidelines on the application of the hemostasis assays in GDM are available.

In Vitro Fertilization

Worldwide, numerous women achieve pregnancy with the aid of IVF. The process involves monitoring and stimulating a woman's ovulatory process, removing egg(s) from the woman's ovaries and letting sperm fertilize them in a laboratory. The fertilized egg (zygote) is cultured for 2 to 6 days in a growth medium and is then implanted in the same or another woman's uterus, with the intention of establishing a successful pregnancy.

VTE incidence is significantly increased in pregnancies after IVF, especially in the first trimester and in the first 6 weeks postpartum.[198,199] The ratio of overall VTE incidence rate during IVF pregnancies compared with reference pregnancies is 3.0 (95% CI: 2.1–4.3).[199] The risk is particularly increased during the first trimester, at 1.5/1,000 after IVF versus 0.3/1,000 (hazard ratio: 4.22, 95% CI: 2.46–7.26). The proportion of women experiencing pulmonary embolism during the first trimester is 3.0/10,000 after IVF versus 0.4/10 000 (hazard ratio: 6.97, 95% CI: 2.21–21.96).[198] The ratios of VTE incidence rate during pregnancy are 2.8 (95% CI: 1.9–4.1) in singleton IVF pregnancies and 4.4 (95% CI: 2.4–8.3) in multiple IVF pregnancies, compared with reference pregnancies. The rate of VTE incidence rate postpartum is 1.2 (95% CI: 0.6–2.8) for singleton IVF pregnancies and 3.9 (95% CI: 1.7–8.8) for multiple IVF pregnancies compared with reference pregnancies.[199]

No strict guidelines for heparin prophylaxis were found for IVF. In fact, the necessity and effectiveness of prophylaxis is under debate and controversial opinions still exist.[200–203]

There is no consensus about the mechanisms of the thromboses in pregnancies after IVF. One possible reason is that women who use IVF have some preexisting thrombotic disorders that led to the infertility.[204,205] Our preliminary data with thrombodynamics support this: no women undergoing IVF in AltraVita clinic in Moscow who had clot growth rate higher than 32.3 μm/min were able to conceive ($n = 13$). In women with clot growth rate less than 32.3 μm/min ($n = 100$), the number of successful pregnancies was 28 ($p < 0.05$) (Balandina et al, unpublished data, 2016). The increase of VTE risk may be due to ovulation induction with human chorionic gonadotropin (hCG) for IVF, which might create a state of hypercoagulability. Supraphysiological increases in estrogen during IVF exert direct effects on individual hemostatic.

For example, a significant increase in the plasma levels of coagulation factors VIII, VWF, and fibrinogen was found between samples drawn before stimulation and at the highest oestradiol levels ($p < 0.002$, $p < 0.002$, and $p < 0.015$, respectively).[107,206–209] Other significant differences after oestradiol stimulation in coagulation factor concentrations included decreases in factor V and nonsignificant decreases in factor II.[107] However, coagulation factor VII activity and antigen decreased significantly.[206] Other authors did not find change in factor VII[208] or these were observed to remain at similar levels before and after follicle stimulation hormone treatment.[107] Similar levels pre/posttreatment were also observed in factors IX and X.[107]

The levels of the coagulation inhibitors PC and AT decreased, while that of free PS increased, after treatment.[107,206,208] The significant increase in the activated protein C (APC) sensitivity ratios during hyperstimulation indicates that acquired APC resistance observed during sex steroid hormone changes in women is at least partially caused by high estrogen levels.[210] TFPI levels were significantly lower in treated patients compared with both case–controls.[107,207]

No significant changes were observed after treatment in the fibrinolytic variables or in those reflecting thrombin activity: F1 + 2, TAT, soluble fibrin, and D-dimers.[206] Other studies, on the contrary, demonstrated that levels of TF, F1 + 2, TAT, plasmin-antiplasmin complexes (PAP), and D-dimer were increased after hCG administration.[207,209] In addition, D-dimer and TAT levels were significantly higher in ovarian hyperstimulation syndrome (OHSS) patients with unsuccessful pregnancy outcome compared with those with successful outcome.[207] The data suggested that a marked hypercoagulability with alterations of TF level is detectable in patients with severe OHSS and that it is related to their clinical outcome.[207] The blood fibrinolytic activity was significantly reduced, as evaluated by an increase in the clot lysis time.[208] The whole blood clotting time was slightly, but not significantly, shortened after ovarian stimulation.[208] Both increased thrombin generation and an increase in OHP from time of downregulation to high-level stimulation were found.[211] These findings demonstrate that IVF treatment is accompanied by the development of a prothrombotic condition.

Cesarean Section

The widespread use of cesarean section (up to 11–25% of all deliveries) draws attention to the risks for this group of patients.[3] The absolute incidence rate of VTE after cesarean section varies from 5.8 to 60 VTEs per 10,000 cesarean sections and depends on the population studied and obstetric practices. The highest rate of thrombosis is observed in the early postpartum period[212,213]; thus, the control of hemostasis before and after the cesarean delivery becomes essential.

The use of low-molecular-weight heparin (LMWH) thromboprophylaxis in low-risk women post–elective cesarean section is still controversial. Current guidelines do not recommend LMWH thromboprophylaxis in this setting without any additional risk factors.[171,214] Currently, there is no randomized control trial addressing the issue of LMWH thromboprophylaxis after elective cesarean section in women with no additional risk factors. A decision analysis study comparing 7-day LMWH thromboprophylaxis with non–post-elective cesarean section suggested that even at low incidence of VTE, benefits of LMWH exceed the risks.[215]

Due to the state of hypercoagulability during delivery, there is lack of major changes after cesarean delivery compared with vaginal delivery, if no additional complications are present. Standard coagulation assays reveal significant differences in fibrinogen concentration between cesarean and vaginal delivery and TEG shows increases in the parameter MCF, which reflects the strength of the clot. No difference was revealed in platelet count, clotting times (PT, APTT), coagulation inhibitors concentration (PC, PS, AT), PFA parameters, and ROTEM parameters.[94] Some authors have identified significant increases in platelet count on day 12 to day 24 of the postnatal period after cesarean section compared with vaginal delivery.[216]

The cesarean section itself does not influence the coagulation state, compared with preoperative state. Although cesarean section is thought to increase the hypercoagulable state, present in pregnancy further, it was found that preoperative TEG parameters were similar to those immediately

Table 5 Changes of coagulation assay parameters in complicated pregnancy

	PE/E	%	Ref	TMA	%	Ref	APS	%	PMF	%	Ref	GSD	%	Ref	IVF	%	Ref	CS	%	Ref	TP	%
Coagulation assays																						
APTT	=		135,137									=		193				=		94		
TT	↑	110	128									=		193								
PT	=		135,137									=		193				=		94		
Thromboelastography/thromboelastometry																						
R/CT	↑/=	140	126,135,149–151	↑	#	158			↑/=	100–140	149,180	=		197				=		94		
K/CFT	↑/=	200	126,135,149–151	↑	#	158			↑↓/=	100–130	149,180	=		197				=		94		
MA/MCF	↑/=	110	126,135,149–151	↓	#	158			=		149,180	=		197				↑	110	94		
CLI30	↓	20	150						=		149,180	=		197								
Thrombin generation test																						
ETP	↑	110–140	136,141,143–145						↑/=	100–120	106,179				↑	115	211					
Peak height	↑	110	141						↑	130	179				↑	120	211					
Lag time	=		141												=		211					
Time to peak	↓/=	90	141,143												↓	90	211					
Overall haemostasis potential																						
OCP									↑	160	178				↑	130	211					
OFP									↑	115	178				↑/=	100–120	208,211					
Markers of coagulation																						
F1 + 2	↑/=	100–260	126,136,144						↑/=	100–110	106,177				↑/=	100–180	206,207,209					
TAT	↑/=	215	136,144						=		106	↑	130	68	↑/=	100–160	206,207	↑	150	217		
FP A																						
D-dimer	↑	125–150	128,140						↑	125–180	106,177	↑	155	68	↑/=	100–570	207	↑	150	217		
PAP															↑/=	220	207					

Abbreviations: APTT, activated partial thromboplastin time; CLI30, clot lysis index after 30 minutes; ETP, endogenous thrombin potential; F1 + 2, prothrombin fragment 1 + 2; FP A, fibrinopeptide A; K/CFT, clot formation time; MA/MCF, maximum clot firmness; OCP, overall coagulation potential; OFP, overall fibrinolysis potential; PAP, plasmin/α_2-antiplasmin complex; PT, prothrombin time; R/CT, clotting time; Ref, reference; TAT, thrombin–antithrombin III complex; TT, thrombin time.

List of symbols: "↑," higher than normal pregnancy values; "↓," lower than normal pregnancy values; "=," no difference between normal and complicated pregnancy values; %, relative to normal pregnancy values; #, a case study, no relative values can be obtained due to the absence of control group, no coagulation occurred (a flat trace in TEG).

Complications: APS, antiphospholipid syndrome; CS, cesarean section; GSD, gestational diabetes mellitus; IVF, in vitro fertilization; PE/E, preeclampsia and eclampsia; PMF, genetic polymorphisms; TMA, thrombotic microangiopathies; TP, thrombocytopenia.

postoperatively.[17] As to markers of coagulation, D-dimer and TAT values are significantly higher in women after cesarean delivery compared with vaginal delivery.[217]

Not only the operative delivery itself but also the procedures of anesthesia and fluid regimens can influence the state of hemostasis. TEG revealed increased hypercoagulation in blood samples from the upper limb 1 hour after a spinal injection,[218] though other authors revealed no change in TEG and coagulation parameters after spinal injection.[219] Spinal anesthesia may influence coagulation parameters also in the lower limbs as it affects the sympathetic innervation and vascular tone of the lower extremities, resulting in vasodilatation, but no clinical evidence to this has yet been obtained. General anesthesia was shown to increase hypercoagulation compared with spinal anesthesia by TEG.[219] Authors explain such changes arise because of tracheal intubation during general anesthesia, which causes the release of catecholamines into blood.[220,221] This procedure can have a stimulatory effect on platelet aggregation.[219] Combined general and epidural anesthesia has been shown to increase significantly the peak velocity, mean velocity, and volume flow in the popliteal vein compared with general anesthesia alone.[222] As to fluid regiments, TEG showed that preloading with 500 mL 6% HES is associated with a mild hypocoagulable effect in healthy parturients presenting for elective cesarean delivery. No significant differences in TEG parameters is seen after preloading lactated Ringer solution with 1,500 mL.[223]

Thrombocytopenia in Pregnancy

Slightly decreased platelet count is believed to be normally associated even with completely healthy pregnancy.[60] More severe cases, classified as thrombocytopenia, are also quite common, and occur in 6 to 10% of all pregnancies, and are the second most common complication of pregnancy after anemia.[224–227]

The leading cause of such maternal thrombocytopenia is gestational thrombocytopenia, that is, "normal" thrombocytopenia associated with fetal development, which is responsible for 75 to 80% of cases and is not usually clinically severe, with platelet counts remaining above 70×109/L. The etiology of gestational thrombocytopenia is unclear, and its diagnosis is based on the exclusion of all other causes of thrombocytopenia. The second most important condition associated with thrombocytopenia is PE (15–20%). Immune thrombocytopenia is the cause of gestational thrombocytopenia in 1 to 4% of the cases, with the rest of pregnancy-specific or nonspecific causes contributing to less than 1%; these include HELLP syndrome, drug-induced thrombocytopenia, APS, infections, and others.

The established diagnostic strategies for thrombocytopenia in pregnancy are aimed at the identification of the cause and evaluation of the severity to make treatment decisions that are, as usual, more complicated and limited in pregnancy. The assays employed (in various combinations) to carry out this strategy include blood count and reticulocyte count, peripheral blood film, liver function tests, thyroid function tests, immunoglobulin level measurement, direct antiglobulin test, aPL, and other mostly nonhemostatic approaches.[224]

It appears that mildly to moderately depressed platelet counts from gestational thrombocytopenia are not associated with any adverse effects to the fetus, neonate, or mother, and no management is necessary other than periodic monitoring. The treatment strategy in patients with immune thrombocytopenia is discussed elsewhere.[227]

We were not able to find any information about either research or diagnostic use of integral hemostasis assays or flow cytometry in pregnancy-associated thrombocytopenia.

Conclusion

Pregnancy significantly shifts the blood hemostatic balance, mostly to the hypercoagulation side, though there are important exceptions, most notably in the placenta.[228] This supposedly physiological mechanism designed by nature to control delivery-related bleeding is presently a major cause of pregnancy-associated prothrombotic disorders. The state of pregnancy increases risks and severity of many of the associated disorders (such as TTP and VTE) and may cause pregnancy-specific disorders (such as HELLP syndrome). The two important pregnancy-associated interventions, IVF and cesarean section, are associated with additional risks of adverse effects related to hemostasis.

Laboratory diagnostics play an important role in timely identification of these disorders and in distinguishing between them (see ►**Table 5**). However, the majority of the assays used in the modern laboratory diagnostics of pregnancy-associated hemostasis disorders do not estimate the degree of pregnancy hemostasis. Except for platelet count and D-dimer level, very few guidelines to patient treatment in pregnancy involve performance of hemostasis assays. This is, in all likelihood, a logical result of the fact that classic assays of hemostasis are not sensitive to the acquired hypercoagulation[15,26] that is predominant in pregnancy. New integral/global assays have demonstrated better sensitivity and hold some promise in this respect. However, additional development and research is sorely needed to obtain the tools necessary to provide predictions of clinically significant events, an outcome that is increasingly becoming realized in many areas of medical science.[229]

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