Section 1 Cellular changes

Section 1 Chapter 1

Cellular changes

Normal hematological changes during pregnancy and the puerperium

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Introduction

There are both subtle and substantial changes in hematological parameters during pregnancy and the puerperium, orchestrated by changes in the hormonal milieu. A thorough understanding of these is important to avoid both over and under-diagnosing abnormalities. Appreciation of the time frame for some of the changes allows sensible planning; this is particularly true when considering thromboprophylaxis.

Some of the quoted reference ranges may differ between centers, depending on laboratory techniques. However, the principles of recognizing physiological changes can still be applied.

Red cells

During pregnancy, the total blood volume increases by about 1.5 l, mainly to supply the needs of the new vascular bed. Almost 1 liter of blood is contained within the uterus and maternal blood spaces of the placenta. Expansion of plasma volume by 25%–80% is one of the most marked changes, reaching its maximum by mid pregnancy. Red cell mass also increases by 10%–20% but the net result is that hemoglobin (Hb) concentration falls.[1] Typically, this is by 1–2 g/dL by the late second trimester and stabilizes thereafter. Women who take iron supplements have less pronounced Hb changes, as they increase their red cell mass proportionately more than those without dietary supplements (the increase is approximately 30% over pre-pregnancy values).[1]

It is hard to define a normal reference range for Hb during pregnancy and the limit for diagnosing anemia. The World Health Organization has suggested that anemia is present in pregnancy when Hb concentration is $<$ 11 g/dL. However, large studies in healthy Caucasian women taking iron supplements from mid pregnancy found Hb values in the early third trimester to be 10.4–13.5 g/dL (2.5th–97.5th centiles)[2]. Studies from other ethnic populations have documented lower third trimester Hb concentrations, which may be attributable to the women entering pregnancy with poor iron stores or with dietary deficiencies of iron and folic acid.

Red cell count and hematocrit (Hct) values are likewise lower in pregnancy, but the other red cell indices change little (Table 1.1), although red cells show more variation in size and shape than in the non-pregnant state. There is a small increase in mean cell volume (MCV), of on average 4 fL for iron-replete women, which reaches a maximum at 30–35 weeks gestation and occurs independently of any deficiency of B12 and folate.[2]

Hemoglobin and hematocrit increase after delivery. Significant increases have been documented between measurements taken at 6–8 weeks postpartum and those at 4–6 months postpartum, demonstrating that this length of time is needed to restore them to non-pregnant values.[1]

Summary points

- Hb concentrations decrease in pregnancy.
- Hb $<$ 10.4 g/dL suggests anemia.
- Hb $>$ 13.5 g/dL is unusual and suggests inadequate plasma volume expansion (which can be associated with pregnancy problems including pre-eclampsia and poor fetal growth).
- MCV is normally slightly increased.
- MCH and MCHC are normally unchanged in pregnancy and do not change with gestation.

The Obstetric Hematology Manual, ed. Sue Pavord and Beverley Hunt. Published by Cambridge University Press.

Table 1.1 Red cell indices during pregnancy and the puerperium

	Gestation			
Red cell indices	**18 weeks**	**32 weeks**	**39 weeks**	**8 weeks postpartum**
Hemoglobin (Hb) g/dL	11.9 (10.6–13.3)	11.9 (10.4–13.5)	12.5 (10.9–14.2)	13.3 (11.9–14.8)
Red cell count $\times 10^{12}$/L	3.93 (3.43–4.49)	3.86 (3.38–4.43)	4.05 (3.54–4.64)	4.44 (3.93–5.00)
Mean cell volume (MCV) fL	89 (83–96)	91 (85–97)	91 (84–98)	88 (82–94)
Mean cell hemoglobin (MCH) pg	30 (27–33)	30 (28–33)	30 (28–33)	30 (27–32)
Mean cell hemoglobin concentration (MCHC) g/dL	34 (33–36)	34 (33–36)	34 (33–36)	34 (33–36)
Hematocrit	0.35 (0.31–0.39)	0.35 (0.31–0.40)	0.37 (0.32–0.42)	0.39 (0.35–0.44)

Mean and reference ranges (2.5th–97.5th centiles). Samples were collected longitudinally from 434 women. Adapted from Ref 2.

White cells

White cell count (WBC) is increased in pregnancy[2] with a typical reference range of 6×10^9–16×10^9/L. In the hours after delivery[3], healthy women have been documented as having WBC 9×10^9–25×10^9/L. By 4 weeks post-delivery, typical WBC ranges are similar to those in healthy non-pregnant women (4×10^9–10×10^9/L).

There has been much discussion about the normal ranges for the different types of white cells.[4] Neutrophils contribute most to the overall higher WBC. There is an increase in immature forms and the cytoplasm shows toxic granulation. The count[3,4] is relatively constant throughout gestation (3×10^9–10×10^9/L), markedly elevated in the hours after delivery (up to 23×10^9/L) and back to non-pregnant values by 4 weeks post-partum (1.5×10^9–6×10^9/L). Neutrophil chemotaxis and phagocytic activity are depressed, the latter being inhibited by factors present in pregnancy serum. There is also evidence of increased oxidative metabolism in neutrophils during pregnancy.

Lymphocyte count[3,4] decreases during pregnancy through first and second trimesters, increases during the third trimester, but remains low in the early puerperium as compared to normal non-pregnant values. Typical pregnancy range for lymphocyte count is 1.1×10^9–2.8×10^9/L, compared with the non-pregnant reference range 0.8×10^9–4.0×10^9/L. Lymphocyte count is restored to normal range by 4 weeks after delivery. Detailed studies of T and B lymphocyte subsets in peripheral blood and the proliferative responses of these cells to mitogens found more helper and suppressor cells and less killer cells during pregnancy. Lymphocyte proliferation in response to a variety of agents was found to be impaired in pregnancy, suggesting that there is an immunosuppressant factor present in the serum.

Monocyte count is higher in pregnancy, especially in the first trimester, but decreases as gestation advances.[4] Typical values[3,4] in the third trimester are 0.2×10^9–1.0×10^9/L, as compared to non-pregnant values 0.1×10^9–0.9×10^9/L. The monocyte to lymphocyte ratio is markedly increased in pregnancy.

Eosinophil and basophil counts do not change significantly during pregnancy.[3]

Myelocytes and metamyelocytes may be found in the peripheral blood film of healthy women during pregnancy and do not have any pathological significance.

Summary points

- WBC is elevated in pregnancy, mostly due to neutrophilia.
- Lymphocyte count is lower and monocyte count higher.
- During pregnancy, only WBC $> 16 \times 10^9$/L is considered abnormal.
- Soon after delivery, only WBC $> 25 \times 10^9$/L is considered abnormal.
- Eosinophil and basophil counts do not change in pregnancy.

Platelets

Large cross-sectional studies in pregnancy of healthy women (specifically excluding any with hypertension) have shown that the platelet count decreases during pregnancy, particularly in the third trimester.[5] This is termed "gestational thrombocytopenia." Almost 12% of women in one study[5] were found to have a platelet count of $< 150 \times 10^9$/L late in pregnancy. Of these women, 79% had platelet counts 116×10^9–149×10^9/L; none had complications related to thrombocytopenia and none of their babies had severe thrombocytopenia (platelet count $< 20 \times 10^9$/L). Thus, it has been recommended that the lower limit of platelet count in late pregnancy should be considered as 115×10^9/L. Only 1% of healthy women have platelet counts $< 100 \times 10^9$/L.

Platelet size is an indicator of the age of the platelets; young ones are large and they become progressively smaller with age. Platelet volume has a skewed distribution, tailing off at larger volumes. The platelet volume distribution width increases significantly and continuously as gestation advances and the mean platelet volume becomes an insensitive measure of platelet size. Studies suggest that platelet lifespan is shorter in pregnancy. The decrease in platelet count and increase in platelet size in pregnancy suggests that there is hyperdestruction of platelets.

Platelet function, as assessed by the time required for whole blood to occlude a membrane impregnated with either epinephrine or adenosine 5'diphosphate (ADP), has been studied in late pregnancy.[7,8] No correlation was found between platelet count and the "closure times" over a range of platelet counts 44×10^9–471×10^9/L in healthy women.[8] Another study found that the closure times were increased in women with severe pre-eclampsia, although they did not correlate with clinical bleeding problems in these women.[9] In women with gestational thrombocytopenia, platelet closure times are influenced by hemoglobin level, being prolonged when there is both thrombocytopenia and anemia.[7] This is perhaps not surprising, given the contribution of red cells to the hemostatic process, in part due to ADP donation. The increase in fibrinogen during pregnancy helps to maintain platelet function.

Summary points

- Platelet count decreases during pregnancy in some patients.
- The lower limit of normal platelet count at term is 115×10^9/L.
- There is evidence of platelet hyperdestruction in pregnancy.
- Platelet closure times are not affected by absolute platelet count in healthy women during pregnancy.
- Platelet closure times are prolonged when there is anemia in addition to a low platelet count.
- The increase in fibrinogen during pregnancy more than compensates for the fall in platelet count.

Coagulation factors

Screening tests used to assess the coagulation pathways include the activated partial thromboplastin time (APTT), which measures the intrinsic pathway, the prothrombin time (PT), which measures the extrinsic pathway, and the thrombin time (TT) which measures the final common pathway. In pregnancy, the APTT is usually shortened, by up to 4 seconds in the third trimester, largely due to the hormonally influenced increase in factor VIII. No marked changes in PT or TT occur.

Many coagulation factors are increased in pregnancy (Table 1.2). Von Willebrand Factor and Factors VII, VIII, X, and fibrinogen increase substantially as gestation advances. In one longitudinal study,[10] Factor VII activity increased from the range 60%–206% (compared to standard) at the end of the first trimester to 87%–336% by term. The same study, found Factors II and V increased in early pregnancy, but then reduced in the third trimester. Another cross-sectional study found a 29% rise in Factor V from 6–11 weeks' to 36–40 weeks' gestation.[11] Increased levels of coagulation factors are mediated by rising estrogen levels and thought to be due to both increased protein synthesis and enhanced activation by thrombin. Coagulation factors remain elevated in the early puerperium and for assessment of true non-pregnant levels, it is best to sample 8–12 weeks after delivery.

Summary points

- APTT is usually shortened in pregnancy.
- Von Willebrand factor and factors VII, VIII, X, and fibrinogen increase.
- There is a variable change in factor XI levels.
- Coagulation factor levels remain high in the early postpartum period.

Table 1.2 Coagulation factors during pregnancy and the early puerperium

	6–11 weeks *N* = 41	12–16 weeks *N* = 28	17–23 weeks *N* = 10	24–28 weeks *N* = 19	29–35 weeks *N* = 36	36–40 weeks *N* = 23	3 days post-natal *N* = 87
Prothrombin fragments 1 + 2 nmol/l	1.1 < 2.9	1.1 < 1.5	1.3 < 2.1	1.8 < 3.4	2.0 < 3.9	1.9 < 3.5	2.2 < 4.9
Fibrinogen activity g/l	3.6 2.5–4.8	3.8 2.5–5.1	3.6 2.6–4.7	4.4 2.9–5.9	4.1 2.5–5.8	4.2 3.2–5.3	4.5 3.1–5.8
Prothrombin activity iu/dl	153 107–200	160 111–209	153 41–265	172 92–252	153 100–211	162 107–217	169 108–231
Factor V activity u/dL	99 39–159	101 39–162	111 47–175	108 50–166	111 43–179	129 65–194	141 71–211
Factor VIII activity iu/dl	107 62–220	129 82–130	189 59–159	187 71–341	180 31–328	176 50–302	192 54–331
Factor IX activity iu/dl	100 49–151	106 82–130	96 74–118	121 59–183	109 65–154	114 79–150	136 65–207
Factor X activity iu/dl	125 88–162	129 78–180	128 50–206	159 52–263	146 81–212	152 113–191	162 69–254
Factor XI activity iu/dl	102 50–154	103 58–147	86 58–114	102 45–162	100 31–169	92 36–181	96 46–146
Factor XII activity iu/dl	137 70–204	160 52–268	186 64–247	170 54–286	178 78–278	179 62–296	174 86–262
Von Willebrand Antigen iu/dl	137 70–204	160 52–268	186 64–247	170 54–286	178 78–278	179 62–296	174 86–262
RCo iu/dl	117 47–258	132 55–298	128 50–206	204 68–360	169 86–466	240 100–544	247 97–630

Mean and 2 standard deviation normal ranges. From a cross sectional study of 239 women, each of whom was only sampled once. Adapted from ref. 11.
RCo: Ristocetin cofactor activity.

Table 1.3 Natural anticoagulant factors during pregnancy and the early puerperium

	6–11 weeks *N* = 41	12–16 weeks *N* = 28	17–23 weeks *N* = 10	24–28 weeks *N* = 19	29–35 weeks *N* = 36	36–40 weeks *N* = 23	3 days post-natal *N* = 87
Total Protein S u/dl	80 34–126	77 45–109	66 40–92	68 38–98	67 27–106	58 27–90	69 37–85
Free Protein S u/dl	81 47–115	72 44–101	64 38–90	60 34–86	54 32–76	57 15–95	58 29–87
Protein C activity u/dl	95 65–125	94 62–125	101 63–139	105 73–137	99 60–137	94 52–136	118 78–157
Antithrombin activity u/dl	96 70–122	100 72–128	100 74–126	104 70–138	104 68–140	102 70–133	108 77–137

Mean and 2 standard deviation normal ranges. From a cross sectional study of 239 women, each of whom was only sampled once. Adapted from ref. 11.

Natural anticoagulants

There are changes in the balance of the natural anticoagulants during pregnancy and the puerperium (Table 1.3). Levels and activity of Protein C do not change and remain within the same ranges as for non-pregnant women of similar age.[11] There are increased levels and activity of Protein C in the early puerperium. Total and free (i.e. biologically available) Protein S levels decrease progressively through gestation. Ranges for total and free Protein S are lower in the

Table 1.4 Natural anticoagulants and markers of fibrinolysis

Number of patients Weeks		41 11–15	48 16–20	47 21–25	66 26–30	62 31–35	48 36–40	61 Post-delivery	61 Post-natal
Fibrin degradation Products μg/ml	**Mean**	1.07	1.06	1.09	1.13	1.28	1.32	1.66	1.04
Fibrinolytic activity (100/Lysis time)	**Mean**	7.6	7.4	7.3	5.5	4.5	5.6	6.75	5.75
Lysis time in hours	**Mean**	13.25	13.5	13.75	18.25	22.25	17.8	14.8	17.4
Antithrombin III:C	**Mean** **Range**	85 49–120	90 46–133	87 42–132	94 47–141	87 42–132	86 40–132	87 48–127	92 38–147
Antithrombin III:Ag	**Mean** **Range**	93 60–126	94 56–131	93 56–130	97 56–138	96 59–132	93 50–136	95 58–133	100 64–134
α_1 Antitrypsin	**Mean** **Range**	124 66–234	136 86–214	125 53–295	146 85–249	149 89–250	154 91–260	172 84–352	77 44–135
α^2 Macroglobulin	**Mean** **Range**	176 100–309	178 98–323	170 92–312	160 88–294	157 85–292	153 85–277	146 81–265	142 82–245

Where no units are shown, values are expressed as per cent of standard. Where shown, range is 2.5th–97.5th centile. Samples were collected longitudinally from 72 women. Post-natal samples were collected 2 weeks-12 months following delivery. The post-natal values were found to be similar to those obtained from healthy pre-menopausal women who were not using oral contraceptives.
Adapted from ref. 10.

first trimester (34–126 and 47–115 iu/dL, respectively) than in women of similar age, not using oral contraceptives (64–154 and 54–154 iu/dL, respectively).[11] This makes it difficult to diagnose Protein S deficiency in pregnancy. Antithrombin levels and activity are usually stable during pregnancy, fall during labor and rise soon after delivery (Tables 1.3 and 1.4).

Acquired activated Protein C (APC) resistance has been found in pregnancy, in the absence of Factor V Leiden, antiphospholipid antibodies or a prolonged APTT.[11] This has been attributed to high Factor VIII activity and may also be influenced by high Factor V activity and low free Protein S levels. Similar acquired APC resistance has been found in women using oral contraceptives and in association with inflammatory disorders. The changes in APC resistance with gestation preclude use of APC sensitivity ratios as a screening test for Factor V Leiden during pregnancy.

Summary points

- Protein C is unchanged in pregnancy.
- Protein S decreases in pregnancy.
- Antithrombin levels decrease during labor.
- There is acquired APC resistance during pregnancy.

Fig. 1.1 Thromboelastograph analyzer.

Thromboelastography

Thromboelastography (TEG)(Fig. 1.1) provides an overall assessment of coagulation by measuring the

viscoelastic properties of whole blood as it is induced to clot in a low-shear environment. The parameters derived from the automated TEG equipment define the reaction time to initiation of a clot (R), the clot formation rate (α) and time (K), the clot strength or maximum amplitude (MA) and clot lysis (reduction in maximum amplitude after 60 minutes, LY60) (Fig. 1.2). The various parameters are correlated and are affected by the availability of fibrinogen and platelet function. The TEG coagulation index (TEG CI) is derived from R, K, MA, and α, which has a normal range of −3 (hypocoagulability) to +3 (hypercoagulability).

In healthy late pregnancy, there is increasing hypercoagulability and the TEG CI has been measured in the range −0.6 to +4.3. Within the first 24 hours of delivery, TEG CI values of −0.5 to +3.9 have been found.[12] The highest TEG CI values have been found during active labor. Parameters return to baseline by 4 weeks postpartum[13] (Fig 1.3). No differences have been found in TEG parameters during pregnancy between smokers and non-smokers. Significantly lower TEG CI values were found in a large study of women who took folic acid supplements[14] during the first trimester (−1.22 to +2.87), indicating that they were less hypercoagulable than those who did not take supplements (−1.52 to +2.60).

Studies of TEG in pregnant women with thrombocytopenia are inconclusive to date. The TEG MA correlates with platelet count as well as fibrinogen, but it is as yet unclear whether TEG parameters can be used clinically to predict the safety of regional anesthetic techniques in women with low platelet counts, especially those with pre-eclampsia.[8,9]

Summary points

- TEG gives a global assessment of coagulation status.
- TEG CI measurement demonstrates the tendency to hypercoagulability in pregnancy.
- There is insufficient experience with TEG in pregnant women with thrombocytopenia or pre-eclampsia to judge its clinical usefulness.

Markers of hemostatic activity

Hemostatic activity can be assessed by measuring markers of both clot formation and clot destruction.[15] Many have been used in research settings, but the ones that have clinical applications are thrombin–antithrombin complexes (TAT) and prothrombin fragments (F 1+2), which reflect in vivo thrombin formation, plus tests that demonstrate plasmin degradation of fibrin polymer to yield fragments, namely D-dimers and fibrin degradation products (FDP). Exact reference ranges depend on the reagents and testing kits used for the assays. Increased levels of F 1+2 are shown in Table 1.2; by term, levels are approximately four times higher than those from a healthy adult population. Likewise, TAT levels[15] increase with gestation; in early pregnancy the upper limit of normal is similar to the adult range of 2.63 μg/L, whereas by term, the upper limit of normal is 18.03 μg/L.

D-dimer levels are very markedly increased in pregnancy, with typical ranges tenfold higher in late pregnancy than in early pregnancy or the non-pregnant state. In one study,[15] where the healthy adult range for D-dimers was < 433 μg/L, by mid pregnancy the range was < 3000 μg/L and by late pregnancy <5300 μg/L. It is thought that the increase in D-dimers reflects the increase in fibrin during pregnancy, rather than increased fibrinolytic activity.

Summary points

- Markers of thrombin production (TAT and F1+2) are elevated in pregnancy.
- D-dimers are tenfold higher in late normal pregnancy than typical levels from healthy non-pregnant women.

Fibrinolysis

There is additional hemostatic control exerted by lysis of the fibrin clot. This is achieved by plasmin, created from plasminogen by activators. The fibrin mesh is lyzed to fibrin degradation products, including D-dimers. Tissue plasminogen activator is the most important endothelial cell derived plasminogen activator. There is reduction in the activity of the fibrinolytic system during pregnancy, mostly due to increased levels of plasminogen activator inhibitors (PAI-1 and PAI-2), which are produced by the placenta. PAI-1 is also produced by platelets and endothelium. There is an exponential

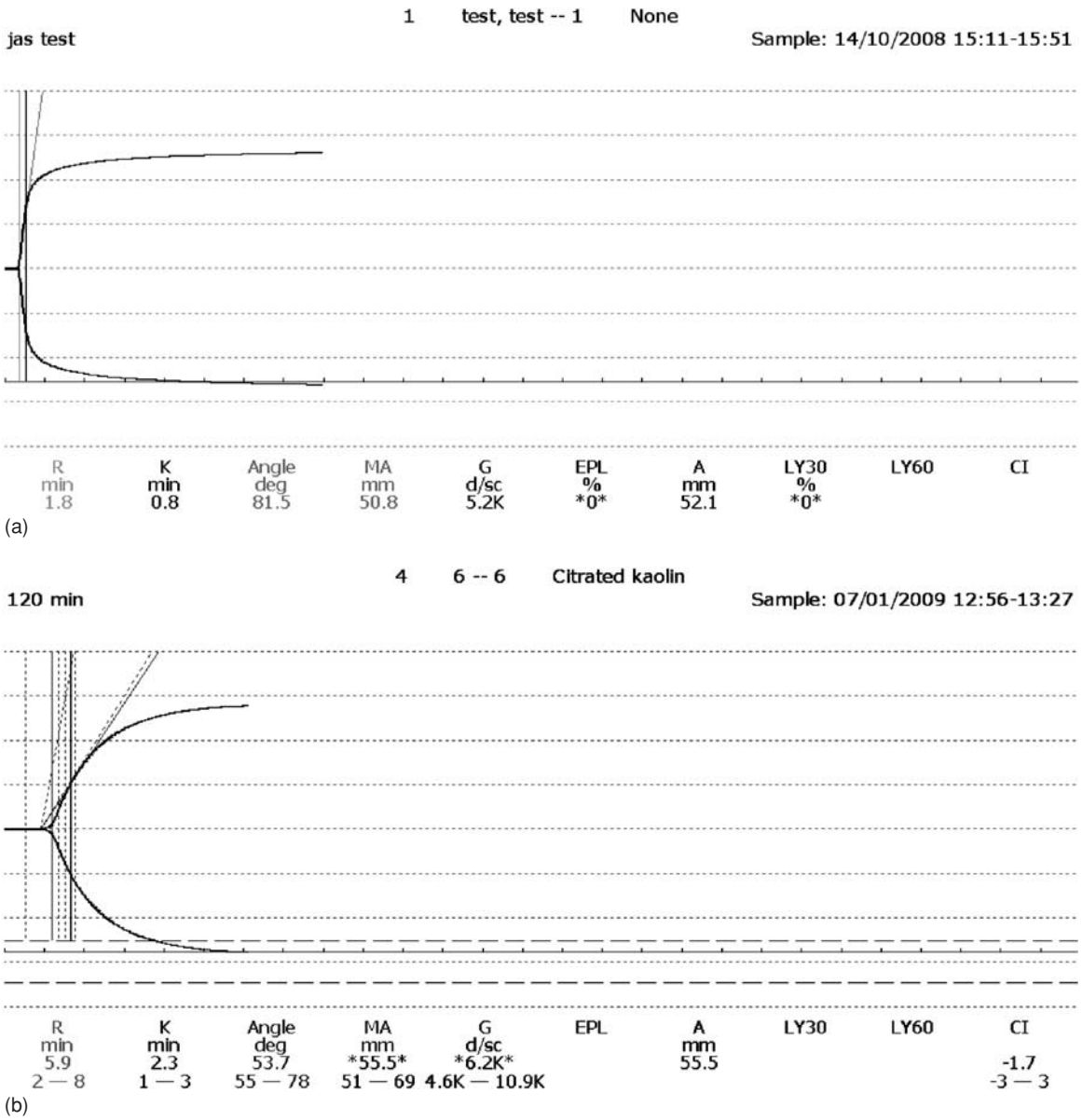

Fig. 1.2 Thromboelastograph trace (a) pregnant (b) non-pregnant, showing shortened R and K times and increased maximum amplitude in pregnancy.

increase in PAI-1 with gestation, from typical values < 50 μg/L in early pregnancy and the non-pregnant state, to values 50–300 μg/L at term.[15] Old studies of fibrinolytic mechanisms in pregnancy and the puerperium demonstrated that levels of plasminogen activator decline through pregnancy, reach their lowest levels during labor and increase soon after delivery.[16] The discovery of PAI-1 and PAI-2 provides the explanation for these changes, which lead to maximum suppression of fibrinolysis during labor.

There are a number of inhibitors of plasmin, including α_2 antiplasmin, antithrombin, $\alpha_1$antitrypsin, $\alpha_2$macroglobulin and C_1-esterase inhibitor. Levels of $\alpha_1$antitrypsin and $\alpha_2$macroglobulin increase after delivery (Table 1.4), as do Factor VIII and fibrinogen

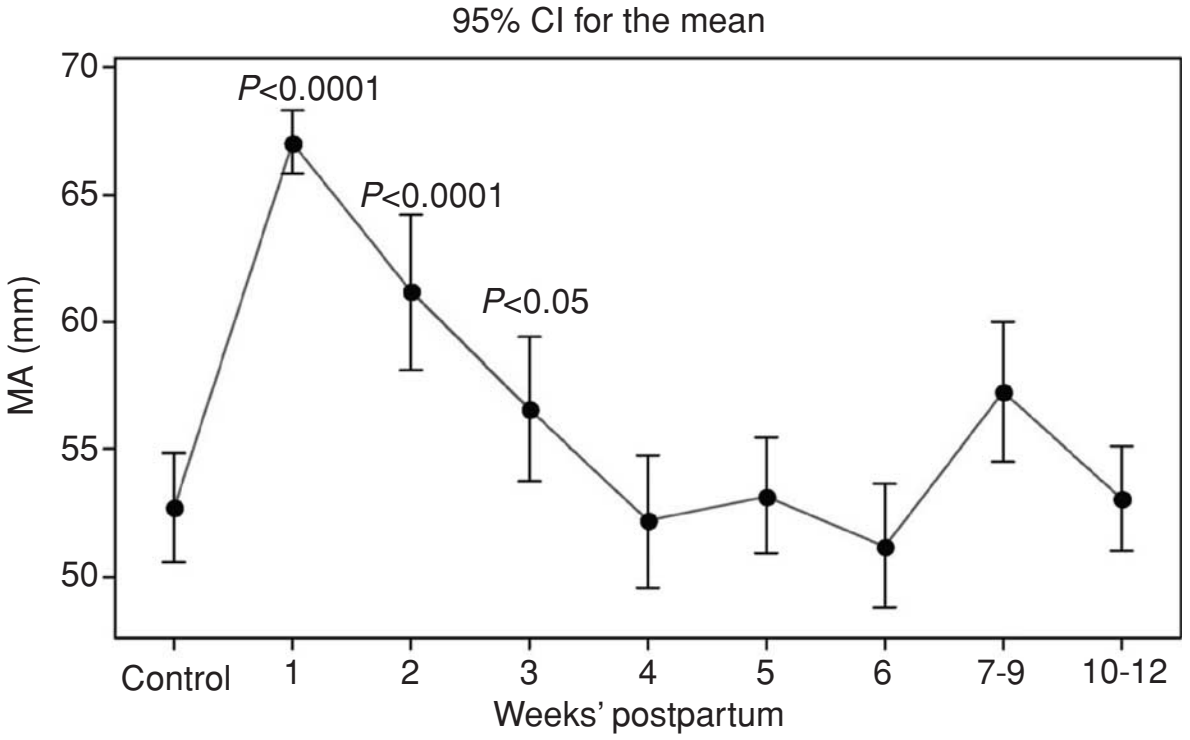

Fig. 1.3 Interval plot of maximum amplitude vs. weeks' postpartum after normal delivery.

activities (Table 1.2); this is an acute phase reaction, similar to that seen after surgery. There are also increased levels of thrombin activatable fibrinolysis inhibitor (TAFI) in pregnancy, which inhibits fibrinolysis by various mechanisms.[17] Overall, although fibrinolytic activity increases after delivery, it takes at least 6 weeks to be completely restored to normal non-pregnant levels.

Clot lysis time is prolonged in pregnancy (Table 1.4), particularly in the third trimester. In one study,[17] the median and interquartile range for clot lysis time was 98 (90–111) minutes in the first trimester, 110 (99–124) minutes in the second trimester and 127 (107–171) minutes in the third trimester, but 92 (80–99) minutes in the first 24 hours after delivery of the placenta.

Increased circulating FDP levels (Table 1.4) and D-dimers[15] are found during pregnancy despite systemic suppression of fibrinolysis. It is thought that there is increased fibrin generation and degradation locally in the placental circulation. Differences have been found in hemostatic and fibrinolytic processes in blood samples from venous placental blood and from forearm blood[10]. It is also possible that clearance of FDP and D-dimers may be altered in pregnancy. Overall, there is a low level of intravascular coagulation, demonstrable from as early as 11–15 weeks gestation.[10] Levels of FDP, D-dimers and soluble fibrin remain high after delivery for at least the first week.

Summary points

- Fibrinolysis is suppressed during pregnancy and especially during labor.
- PAI-1 from endothelial cells is increased in pregnancy.
- PAI-2 is produced in the placenta.
- Various factors continue to suppress fibrinolysis soon after delivery.
- Raised FDP and D-dimers indicate clot formation and destruction, possibly locally in the placental circulation.

Homocysteine

Homocysteine levels fall in early pregnancy and are significantly reduced compared to the non-pregnant state, in all three trimesters.[18] This appears to be multifactorial and related to the hormonal changes in pregnancy, physiological hemodilution, increased renal clearance of homocysteine, folic acid supplementation and enhanced remethylation of homocysteine due to increased demands for methionine by the fetus.