**Haemostasis**

* Hemostasis is a highly balanced interaction between blood vessels, platelets, and soluble factors in the formation and dissolution of blood **clots**. These interactions maintain blood in a fluid state that is necessary for the normal function of blood.
* Haemostasis depends on:

1. Normal structure and function of the blood vascular system.
2. Normal numbers and function of platelets.
3. Normal coagulation system.
4. Stability of the clot.

* Haemorrhage secondary to either defects in the numbers and function of platelet, or defects in the coagulation system, are common problems.
* Following vascular injury, the process of hemostasis promotes the rapid formation of **platelet plugs** and **clots** at the site of injury to minimize blood loss.
* The formation of a **platelet plug** over the injury site is call (primary haemostasis) and the stabilization of this plug by a mesh (net) of **fibrin** formed is call (secondary haemostasis).
* Coagulation is the process that results in generation of **thrombin**, (it is a multifunctional plasma enzyme that converts soluble fibrinogen into insoluble fibrin).
* Once the blood vessel is repaired generation of **plasmin**,(it is plasma enzyme, dissolution of fibrin via fibrinolysis) occure and the clot dissolves , re-establishing normal blood flow.
* There is a constant balance between clot formation and clot dissolution (**fibrinolysis**)
* Disorders of hemostasis or unbalanced hemostasis may lead to **hypocoagulation** (hemorrhage) or **hypercoagulation** (thromboembolic disorders).
* The processes of **coagulation** and **fibrinolysis** also are involved in tissue inflammation and repair, and tumor metastasis.

**PLATELETS**

**A. MORPHOLOGY**

* Mammalian platelets are small, anuclear, cytoplasmic fragments of megakaryocytes. They average 2-5 μm in diameter in most species and have fine reddish granules. Feline platelets are more variable in size and may be as large as erythrocytes. **B.PRODUCTION**
* Platelets are produced by extension of megakaryocyte cytoplasm into vascular sinuses within bone marrow. Proplatelets fragment into individual platelets in circulation.
* **Thrombopoietin (TPO)** is a key humoral regulator of platelet production.
* Severe thrombocytopenia can result in enhanced production of TPO by bone marrow cells,and Inflammatory conditions can result in enhanced TPO production by hepatocytes, mediated by **IL-6**.
* Platelet circulating lifespan is approximately 5-9 days in most animal species.

**C.FUNCTION**

1-Have important role in thrombosis.

2- Have important role in tissue inflammation.

3- Involved in tissue repair.

**PRIMARY HAEMOSTASIS**

**Primary** haemostasis is the interaction between platelets and damaged vascular endothelium to form a platelet plug at the site of injury. Primary haemostasis disorder appear as mucosal or cutaneous petechiae, ecchymoses and purpura (thrombocytopenia common).

* excessive bleeding from mucosal surfaces (haematuria, epistaxis, melaena, uterine or gingival haemorrhage) or excessive bleeding after surgery or trauma (including clipping of claws). These signs can be seen in any disorder of primary haemostasis.
* Vascular defects, von Willebrand's disease (vWD) and defects in the numbers and function of platelet result in disorder primary haemostasis.

**Components required for primary haemostasis**

1. Endothelium

* Collagen and von Willebrand factor are involved in platelet adhesion to endothelium at sites of vascular damage.

1. Platelets

* Mammalian platelets are small anucleate cells 2-5µm in diameter derived from megakaryocytes in the bone marrow. They have a plasma membrane (cytoskeleton) that contains numerous types of **receptors** and **contractile proteins** that allow them to change shape and various types of **granules**.
* Platelets have a phospholipid bilayer membrane that contains glycoproteins. These glycoproteins serve as **receptors** for activation, adhesion, and aggregation.
* The **granules** contain cations, nucleotides, amines, fibrinogen, vWf and factors that promote vascular repair. There are three types of membrane-bound cytoplasmic granules:

1. **Alpha granules**
2. **Dense granules**
3. **Lysosomes**

* Platelets do not adhere to intact healthy endothelium because:

1-Intact endothelial cells secrete antithrombotic substances such as **prostacyclin**.

2- Intact endothelial cells have **negatively charged surface** repel platelets.

**EVALUATION OF PRIMARY HAEMOSTASIS**

* A primary haemostatic plug provides a temporary close over the injured site in the blood vessel.
* Vascular defects, von Willebrand's disease (vWD) and defects in the numbers and function of platelet result in disorder in primary haemostasis.

**1-Buccal mucosal bleeding time**

The bleeding time is the time it takes for bleeding from a standardized superficial incision to stop. In animals, the buccal mucosal bleeding time (BMBT) is the most reliable method for measuring bleeding time.

**2- Estimation of platelet number and platelet morohology**

Platelet numbers can be estimated on examination of stained blood smears. Each platelet in an oil immersion lens field represents about 15 x l09 platelets/l.

**3-Manual platelet count**

Manual platelet counts are simple to perform but have an inherent coefficient of variation of 20-25%, even when performed by experienced personnel.

**4- Clot retraction**

Ablood sample collected without anticoagulant from a normal animal will clot in the tube within minutes. The contractile proteins in platelets then contract, squeezing the serum out of the clot, resulting in separation of the clot from the serum. Abnormal retraction may be due to hypofibrinogenaemia, thrombocytopenia and thrombopathia.

* **Platelet function tests** is indicated when there is clinical evidence suggestive of defective primary heamostasis and/or a prolonged BMBT, but thrombocytopenia and vWD have been ruled out.

**1-Platelet aggregometer**: used to the ability of the platelets to attach together (aggregate) and release the contents of their granules.

**SECONDARY HAEMOSTASIS**

Secondary haemostasis is the process of blood coagulation. Disorders of secondary haemostasis, such as haemophilia Aor anticoagulant rodenticide toxicity, cause larger areas of hemorrhage, such as haematoma, body cavity haemorrhages (haemothorax, haemoperitoneum).

* In coagulation the generation of thrombin converts soluble fibrinogen to insoluble fibrin.
* The fibrin is then crosslinked and forms a mesh, which stabilizes the platelet plug formed in primary haemostasis.
* Thrombin formation is the product of a process of enzymatic reactions initiated by tissue trauma and release of tissue factor.
* **Fibrinogen** **:** It is a large plasma protein synthesized by the liver. preventing spontaneous aggregation of platelets within the circulation due to their highly negatively charged fibrinopeptides. Thrombin produced in the coagulation process cleaves the negatively charged fibrinopeptides from the fibrinogen molecule to form fibrin monomers, which then spontaneously aggregate to form fibrin.
* **Role of Vitamin K in Haemostasis:** Four of the major coagulation proteins, factors II (prothrombin), VII,IX and X, depend on vitamin K for functional clotting activity , as do several proteins with anticoagulation activity, namely proteins C and S. This group of vitamin K-dependent coagulant and anticoagulant proteins are known as the **prothrombin complex**. Like most other clotting factors, the proteins in the prothrombin complex are manufactured by the liver. The proteins are manufactured by hepatocytes in an inactive precursor form and to become functional must contain the amino acid (glutamic acid) in its carboxylated form. Carboxylation of glutamic acid in the proteins of the prothrombin complex requires the presence of vitamin K. In the absence of vitamin K, these proteins are only present in non-functional forms.
* **Hypoprothrombinemia** occur in**:**

Dietary deficiency of vitamin K.

Inadequate absorption of vitamin K.

Impaired formation of prothrobin by liver eg: infectious canine hepatitis.

* **INHIBITORS OF COAGULATION**

1. Antithrombin III (ATIII).
2. Tissue factor pathway inhibitor.
3. Vitamin K-dependent anticoagulant plasma proteins (proteins C and S).

**EVALUATION OF SECONDARY HAEMOSTASIS (coagulation)**

In secondary haemostasis the primary haemostatic plug is stabilized by the formation of cross-linked fibrin. Primary and secondary haemostasis must occur for effective haemostasis to take place.

**Tests of secondary haemostasis**

**1-Activated clotting time**

The activated clotting time (**ACT**) is a simple and useful screening test that measures the time it takes for fresh whole blood to clot in the presence of a substance that initiates the contact activation of coagulation.

**2-Whole blood clotting time**

Similar to the ACT, the WBCT will be prolonged in severe thrombocytopenia and hypofibrinogenaelnia.

**3-The prothrombin time :**The PT is sensitive to defects in the extrinsic pathway (factor VII) and/or factors in the common pathway (fibrinogen, II, V and X).

**4-The activated partial thromboplastin time (APTT**) identifies coagulation abnormalities of the intrinsic and common pathways.

**Fibrinolysis**

The end product of coagulation is the formation of insoluble fibrin. The next step in the process is the repair of the damaged blood vessel, lysis of the clot and restoration of blood flow. This is achieved through dissolution of the clot by the fibrinolytic system. Both fibrinogen and fibrin are digested by the enzyme **plasmin**, which is derived from an inactive precursor (**plasminogen**) and activated by several different molecules, of which the most important is **tissue plasminogen activator (tPA**).

**Laboratory tests of fibrinolysis**

**1-Thrombin clot time**

**Haemostasis Disorder**

* **von Willebrand's disease (vWD)** is caused by a deficiency of, or abnormality in, a large plasma glycoprotein called von Willebrand factor (vWf). It is the most common inherited disorder of haemostasis in dogs and has been diagnosed in cats.
* **Haemophilia**, haemophilia, results from deficiency in the factor VIII (haemophilia A) that reported in a large number of species (dogs, cats, horse, sheep and cattle), while deficiency in the factor IX (haemophilia B) that reported only in a few species (dogs and cats).
* **Immune-mediated thrombocytopenia (IMT)** is a disease in which antibodies bound to the surface of platelets result in premature platelet destruction by macrophages. IMT may occur alone or in association with systemic lupus erythematosus (SLE); rheumatoid arthritis; neoplasia; viral, bacterial, rickettsia1 or parasitic infections; or drug administration.

**Laboratory evaluation of thrombocyte (platelet) count**

1. Peripheral blood smear evaluation. 3-4 platelet/oil immersion field indicates thrombocytopenia(about 15x109/l).
2. Platelet count by automated haemtology analyser.
3. Mean platelet volume by automated haemtology analyser.
4. Bone marrow evaluation in case of impaired platelet production.

**Thrombocytopenia**

**The spleen** normally contains up to 30-40% of the circulating platelet mass.Epinephrine induced splenic contraction from excitement, fear, pain, or exercise may increased platelet counts and cause thrombocytosis. Conversely, splenic congestion or hypersplenism may sequester platelets to cause thrombocytopenia.

Common clinical signs with thrombocytopenia include: epistaxis, haematochezia, melaena, hematuria, continuous hemorrhage or oral cavity hemorrhage. Causes ofThrombocytopenia:

1. Decreased in platelet production (bone marrow disease and haemorrhage) accompanied by: leukopenia and non-regenerative anemia.
2. Accelerate platelet destruction eg.IMT.
3. Platelet sequestration:

1-neoplasia: eg.MPD , LPD

2-hematoma

3-splenitis

4-hypothermia and sepsis

4- Infectious agents

* 1. Late stages of ehrlichiosis and other rickettsial diseases frequently are associated with persistent thrombocytopenia.
  2. FeLV, FIV, equine infectious anemia (EIA) virus, , and bovine viral diarrhea (BVD) virus.

**Thrombocytosis**

1-Splenic contraction: exercise, excitement and acute blood loss.

2-Post-splenectomy thrombocytosis condition.

3-Reative thrombocytosis: inflammation , infection.

4-Post thrombocytopenia.

5-Primary thrombocytosis: platelet leukemia and polycythemia vera.