Angiogenesis = growth of blood vessel sprouts from capillary blood vessels

Depends on angiogenic factors and their inhibitors

Angiogenesis is required for

- growth of many normal tissues
- repair of many damaged tissues
- increase in adipose tissue
- tumour growth

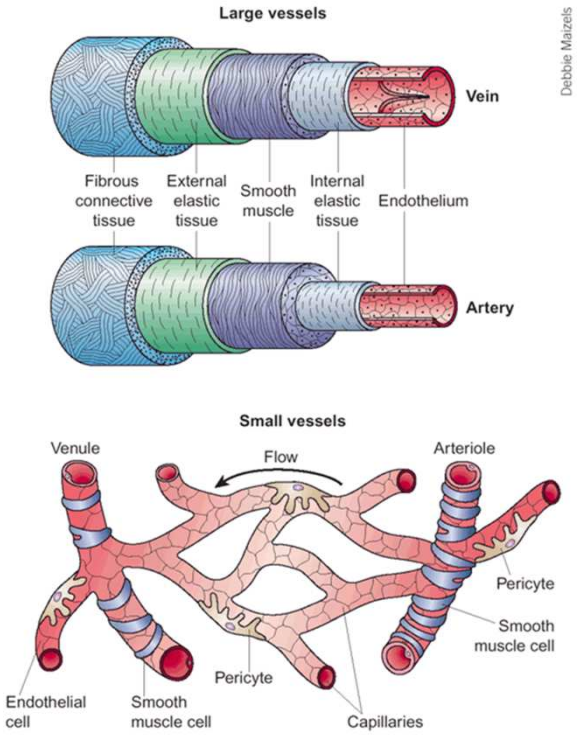
Healing
Growth

Folkman J (2007) Nature Reviews/ Drug discovery. April 6:273-286

- The modern history of angiogenesis began with the work of **Judah Folkman**, who hypothesized (and published in 1971) that tumor growth is angiogenesis-dependent.

Blood vessel structure

- Focus on capillaries:
  - –structure varies between tissues
- Endothelial cells
- Pericytes
- Smooth muscle cells



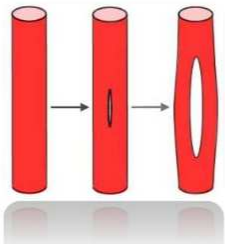
Regolation of angiogenesis

A. Metabolic Factors

- Capillary growth is proportional to metabolic activity.
- Increasing metabolic activity stimulates blood vessel growth.
- Decreasing metabolic activity causes vascular regression.
- Long-term increases in blood pressure lead to vascular rarefaction.
- Oxygen is a master signal in growth regulation of the vascular system.
- Role of adenosine in metabolic regulation of vascular growth

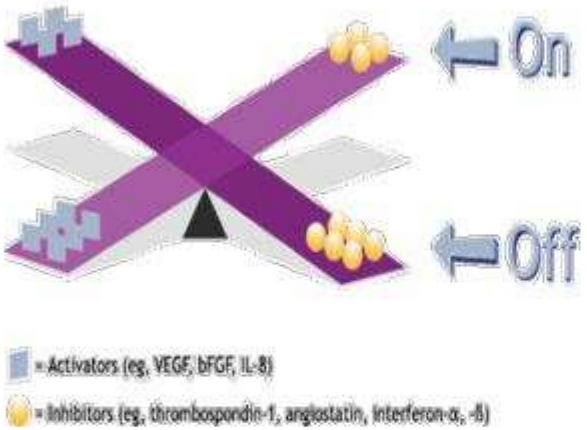
B. Mechanical Factors

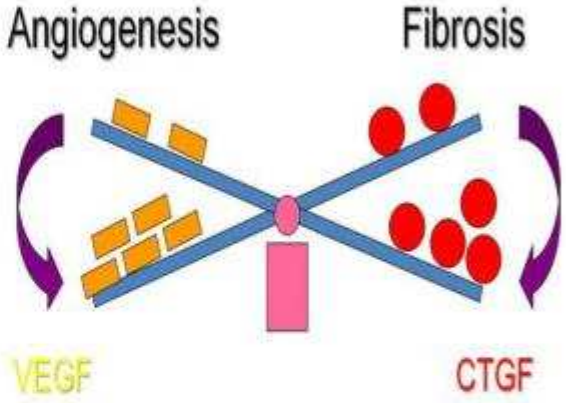
- Regardless of the growth factor(s) that stimulate angiogenesis, the fundamental steps required to build new capillaries are essentially the same.
- A better understanding of the mechanosensory mechanisms could therefore provide the basis for unique therapeutic interventions to control angiogenesis.



Shear stress-induced intussusceptive angiogenesis gives rise to longitudinal splitting of blood capillaries.

The balance hypothesis of the 'angiogenic switch'.





Angiogenesis is tightly controlled by the balance of two sets of counteracting factors – angiogenic activators and inhibitors.

VEGF

Vascular Endothelial Growth Factor

Originally described as endothelial **cell-specific mitogen** (Abraham and Schilling, 1989);
Now as VEGF and also known as **vascular permeability factor (VPF).**

VEGF is a sub-family of growth factors, to be specific, **the platelet-derived growth factor family of cystine-knot growth factors.**

Native VEGF is **a basic,** heparin-binding, homodimeric **glycoprotein of 45 kDa** (Ferrara, 1992).

Important signalling protein .

Mainly involved in **angiogenesis** and **vasculogenesis** .

Tumor cells , macrophages, platelets, keratinocytes, and renal mesangial cells etc .

VEGF plays a role in normal physiological functions such **as bone formation, hematopoiesis, wound healing, and development**. (Tischer and Vaisman 1990).

Historical perspectives

VEGF - First identified in guinea pigs, hamsters, and mice (Senger *et al.* in 1983)

Purified and **cloned** (Ferrara and Henzel , 1989)

Tischer *et al.* (1991) – discovered alternative **splicing of** VEGF

Crystal structure of VEGF- first **at 2.5 Å resolution** and later **at 1.9 Å** Discovered by Christinger and De Vos (1996-1997)

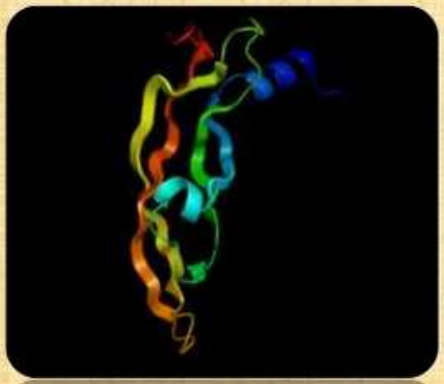
Classification of VEGF

Mammals – Five classes

- VEGF-A
- VEGF-B
- VEGF-C
- VEGF-D
- PGF

Viruses - VEGF-E

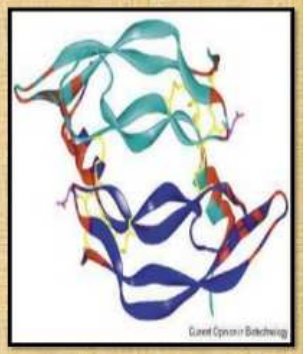
Snake venom - VEGF-F



Crystal structure of Vammin, a VEGF-F from a snake venom

VEGF – A (Senger *et al.*, 1983)

- Consist of **121, 165, 189** and **206** peptides in humans.
- Main **isoforms**- 121 &165.
- Found in chromosome **6** in **human** and **11** in **rats.**
- Up regulate -**nitric oxide** production.



Oloffson et al., 2000

Angiogenesis

VEGF = ONLY mitogenic for endothelial cells

FGF = mitogenic for many cell types.

Angiogenesis

↑ **Migration of endothelial cells** (Bates and Harper, 2003)

↑ **Mitosis** of endothelial cells

↑ **Activity of Matrix metalloproteinases (MMPs)** (Garrido-Urbani *et al*. 2008).

↑ **αvβ3 integrin activity** (Sato 2001).

VEGF-B

Consists of 188 amino acids.
Present on **chromosome 6 in humans (Olofsson, 1996).**

Regulates Embryonic angiogenesis
(Creates new blood vessels during embryonic development)

Promotes fatty acid uptake in endothelial cells, which is critical in organs with high metabolic stress such as the heart **(Hagberg *et al*. 2010).**

Contributes to formation of muscular tissue in the heart;
Protects neurons in the cerebral cortex and retina during a stroke.

VEGF-C

Encoded by the ***VEGFC*** **gene** in humans and **located on chromosome 4q34** (*Paavonen et al.1995).*

Regulates Lymphangiogenesis- Creates new lymph vessels and plays a role in vascular **endothelial cell growth, production, and transition** (Alitalo *et al.,* 2002).

Important for **neural development,** and **blood pressure** regulation.

Closest **structural and functional relative of VEGF-D**.

VEGFC/VEGFR3 are critical regulators of lymph endothelial function. Loss-of-function **VEGFR3 mutants** in humans cause **lymphedema** (Maes *et al.,* 2002)

VEGF-D

The *VEGF-D gene contains* ***seven exons*** *and is found on* the **X chromosome** (Gera Neufeld *et al*., 1999).

Function - Development of **lymphatic vasculature surrounding lung bronchioles.**

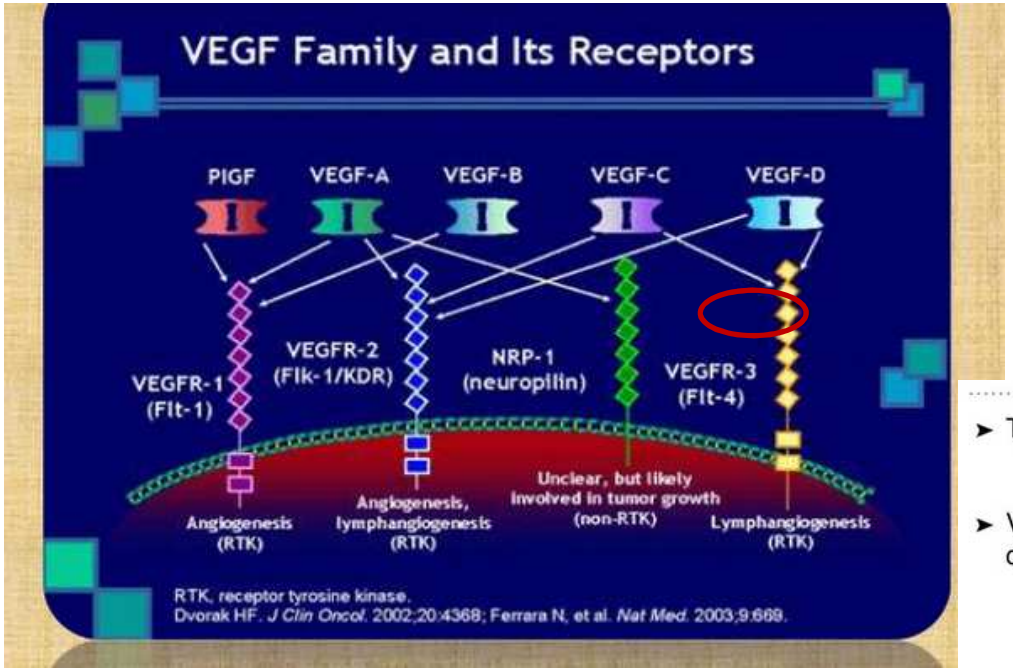
Its **expression correlates** with lymph node metastasis in colorectal , lung and ovarian carcinomas .

Receptors & Mechanism of action of VEGF

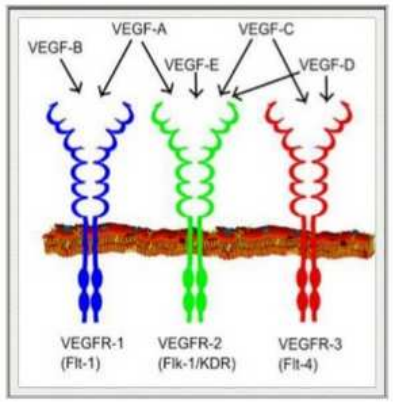
VEGF is a diametric glycoprotein and **acts via tyrosine kinase receptors 1 ,2 3, and NRP 1 & 2** receptors; predominantly **located on endothelial cells** (Devries *et al.,* 1992)

All members of VEGF family stimulate cellular responses by binding to tyrosine kinase receptors on cell surface, **causing them to dimerize and become activated through transphosphorylation.**

Neuropilin-1 and Neuropilin-2 co-receptors, which bind selectively **VEGF165** that encode VEGF specific tyrosine-kinase receptors.



- Tyrosine kinase receptors
- VEGF-2 mediates all cellular responses



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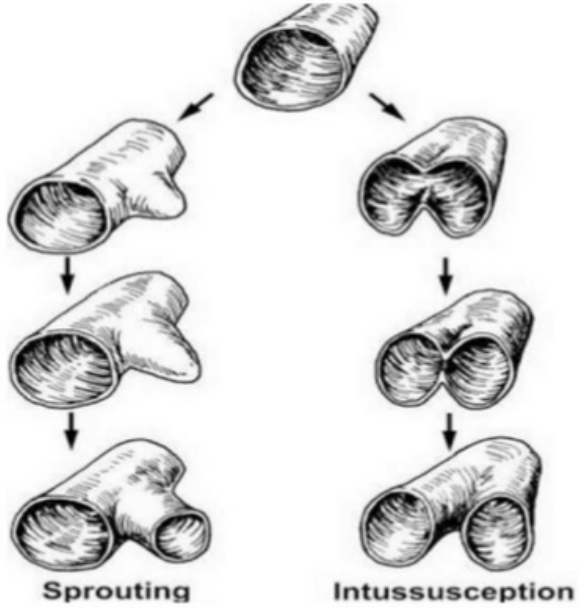
VEGF RECEPTORS

- Growth factors of the VEGF family exert their biological effect via interaction with receptors located on endothelial cell membranes.

- Three receptors have been identified that bind different VEGF growth factors: VEGFR1 , VEGFR2 and VEGFR3

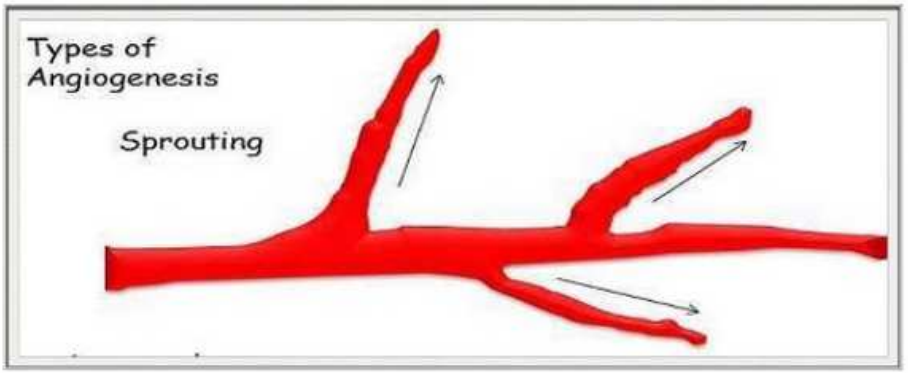
- These receptors belong to the superfamily of receptor tyrosine kinases (RTK)

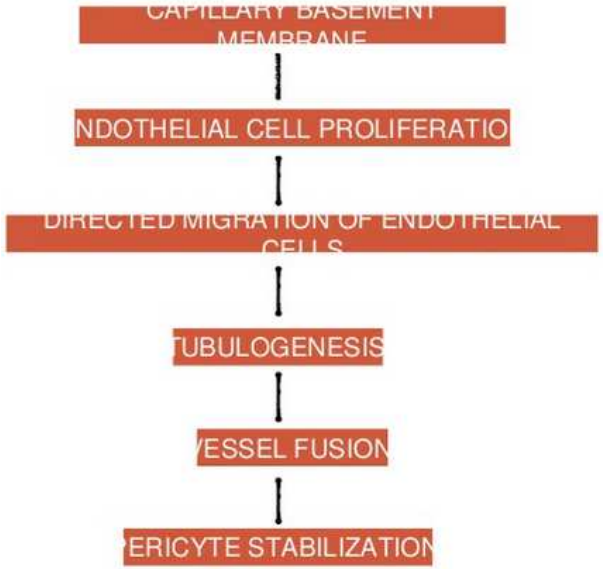
- They are transmembrane proteins with a single domain


Sprouting
Intussusception

SPROUTING ANGIOGENESIS

- Sprouting angiogenesis is characterized by sprouts composed of endothelial cells
- They grow towards an angiogenic stimulus such as VEGF-A.
- It can add blood vessels to portions of tissues previously devoid of blood vessels.
- It is initiated in poorly perfused tissues

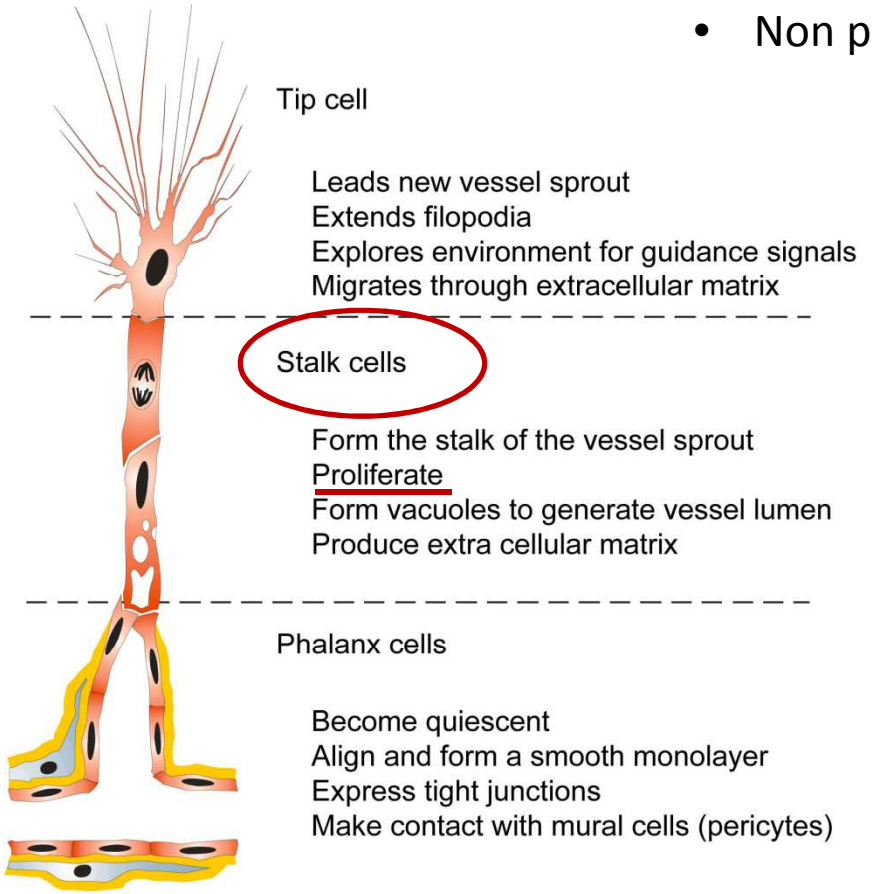




- basal membrane is degradate and tip cells project into extracellular matrix
- gradient to guide endothelial cells projection

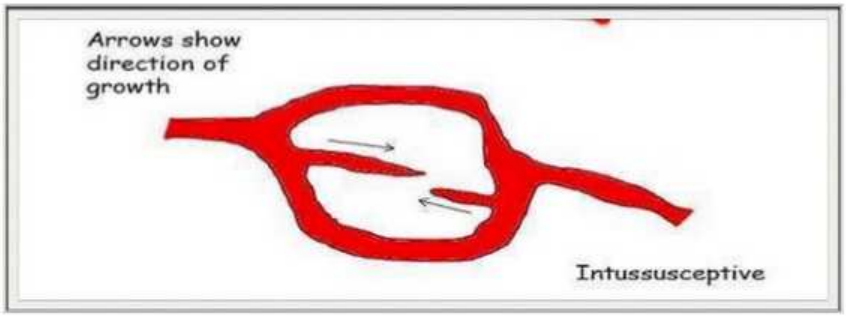
Tip cells

- Apex of sprout
- Hightly motile
- Exend numerous filopodia
- Tubeless
- Non proliferative phenotypes



The angiogenic process, as currently understood, can be summarized as follows:

- A cell activated by a lack of oxygen releases angiogenic molecules that attract inflammatory and endothelial cells and promote their proliferation.
- During their migration, inflammatory cells also secrete molecules that intensify the angiogenic stimuli.
- The endothelial cells that form the blood vessels respond to the angiogenic call by differentiating and by secreting matrix metalloproteases (MMP), which digest the blood-vessel walls to enable them to escape and migrate toward the site of the angiogenic stimuli.
- Several protein fragments produced by the digestion of the blood-vessel walls intensify the proliferative and migratory activity of endothelial cells, which then form a capillary tube by altering the arrangement of their adherence-membrane proteins.
- Finally, the capillaries emanating from the arterioles and the venules will join, thus resulting in a continuous blood flow.



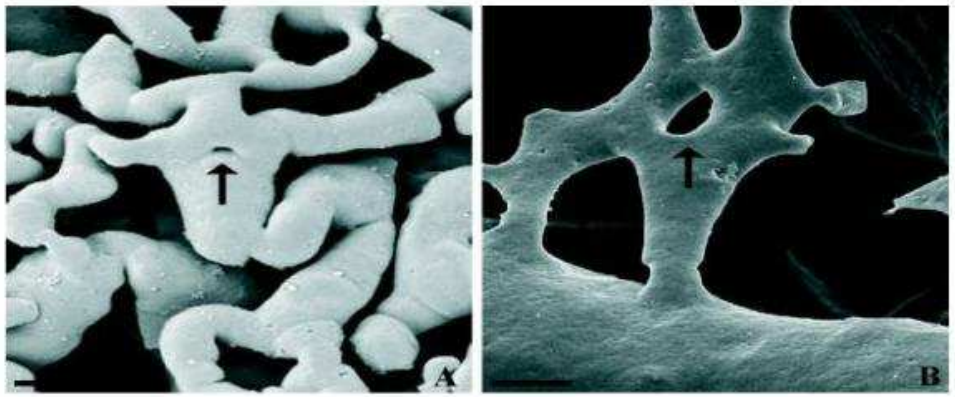
INTUSSUSCEPTIVE ANGIOGENESIS

- Intussusceptive angiogenesis is also called splitting angiogenesis
- The vessel wall extends into the lumen causing a single vessel to split in two
- . This type of angiogenesis is thought to be fast and efficient compared with sprouting angiogenesis:

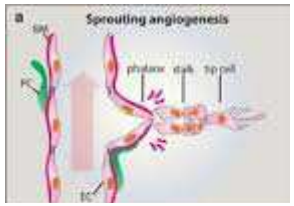
1. Reorganization of existing endothelial cells
2. No immediate endothelial proliferation
3. Migration

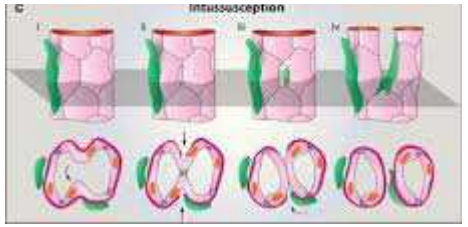
- occurs in the virtual absence of endothelial cell proliferation
- requires only 4–5 h for completion
- is present only in structures in rapid neovascularization

Intussusceptive Angiogenesis



Pillars are non-sprouting angiogenesis features





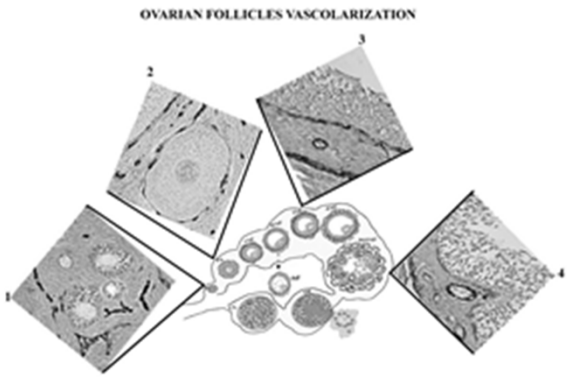
Endothelial cells projections are believed to contein contractile and shape consistent with filopodia

Basement membrane is degraded and tip cells project into the extracellular matrix

Basement membrane <u>is not degraded</u>. Endothelial cell projections are <u>not oriented into the extracellular matrix</u> (across the vessel lumen)

gradient to guide endothelial cell projection

No gradient to guide endothelial cell projection

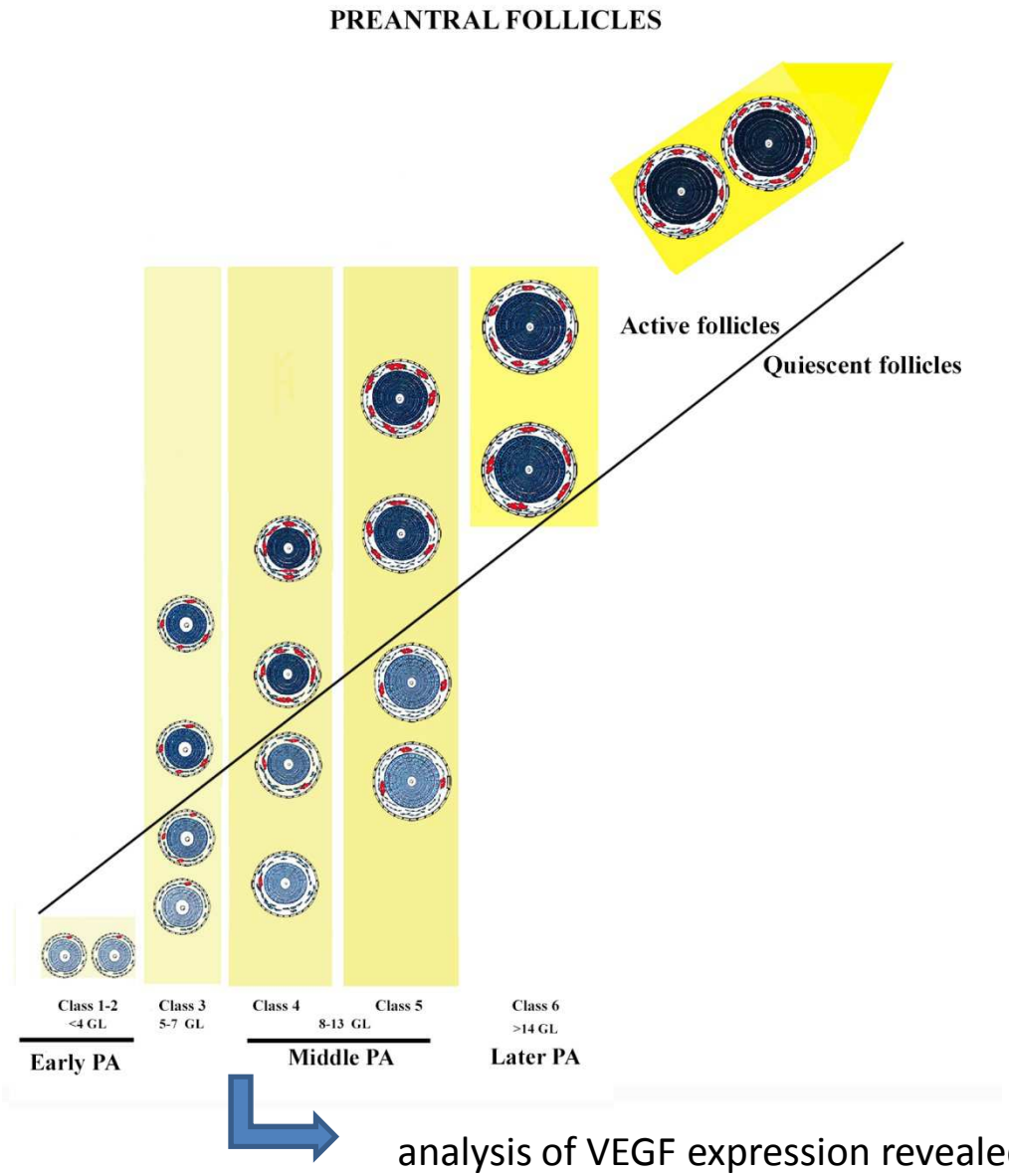


Primordial and primary follicles do not have a specific vascular network

Vascular preantral follicle is organized:
- a ring of blood vessels near to basal membrane
- spots of vessel at the periphery of basal membrane

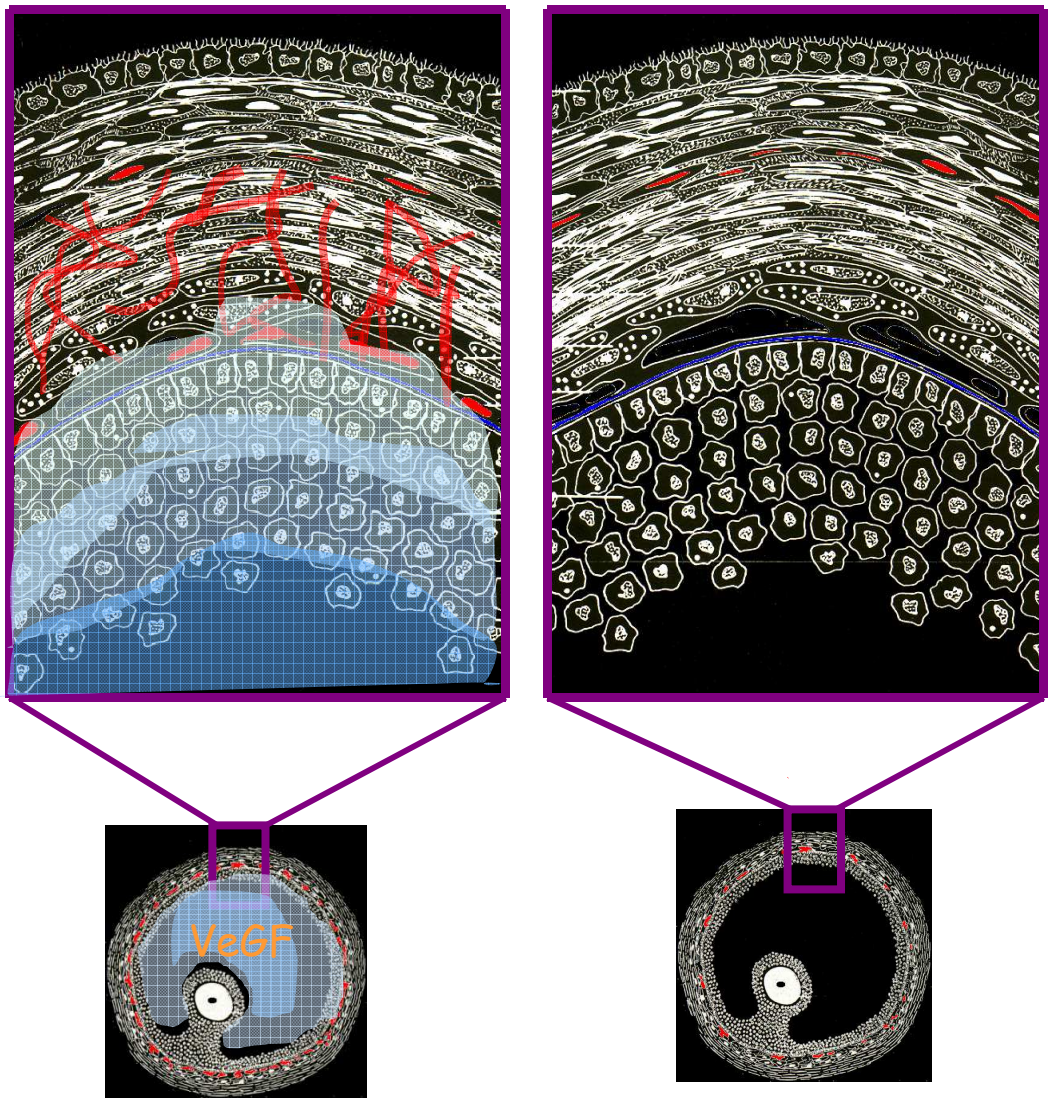
Antral follicles present two concentric blood vessel networks connected to each other by anastomotic vessels

In atretic follicles the the first vascular network that is reduced the inner network



analysis of VEGF expression revealed that the angiogenic stimulus progressively increases passing from class 3 to classes 4-5 preantral follicles

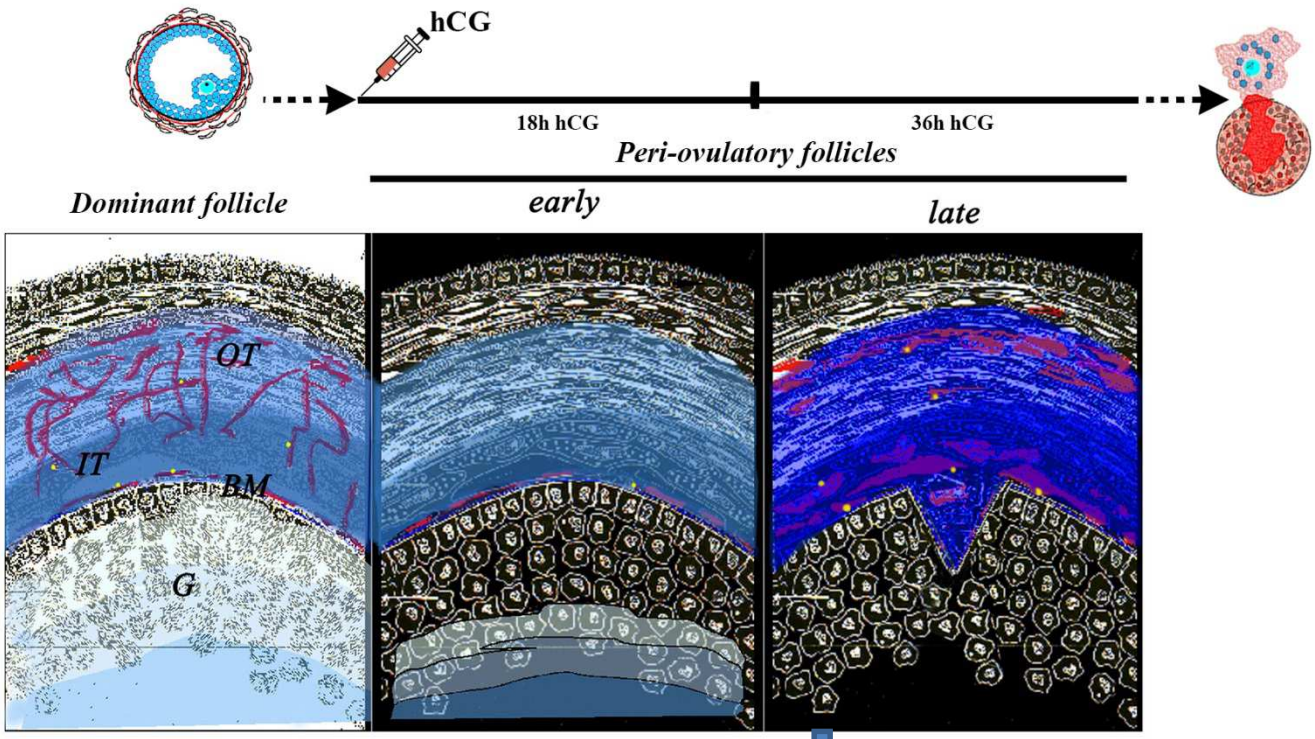
Antral Follicle Atretic Follicle



The persistence of active inner capillary network evidenced the importance of the a correct trophic supply of oxygen and metabolites both to the avascular granulosa compartment and to the germinal cells

VEGF in follicular fluid suggest a different solubility of the protein secreted

PERIOVULATORY PHASE



Late periovulatory follicles

- acquiring an undulated aspect
- present non sprouting and sprouting angiogenesis