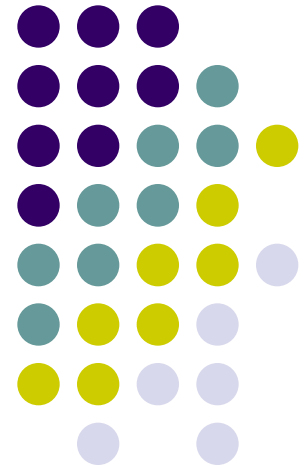


UNDERSTANDING UTERINE BLEEDING

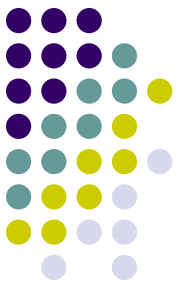
Margaret Rees

Reader in Reproductive Medicine, University of
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and University of Turku
Editor in Chief Maturitas



Competing interests: none declared

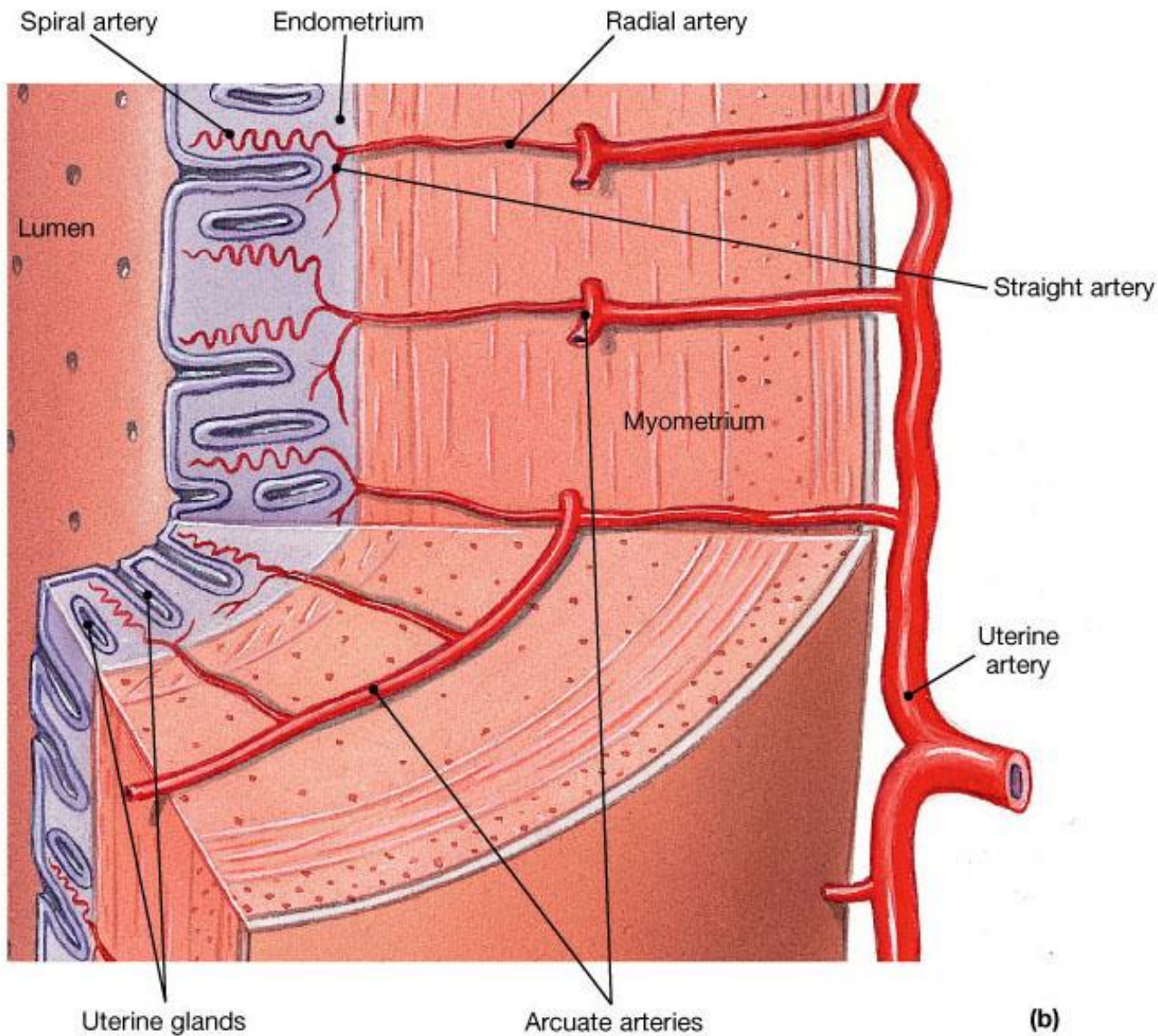


What is menstruation

- A periodic discharge of sanguinous fluid and sloughing of the uterine lining in the female
- An event characteristic of the reproductive cycle in humans and most subhuman primates

Scommegna and Dmowski1997





(b)

Menstrual taboos



- “Contact with the monthly flux of women turns new wine sour, makes crops wither, kills grafts, dries seeds in gardens, causes the fruit of trees to fall off, dims the bright surface of mirrors, dulls the edge of steel and the gleam of ivory, kills bees, rusts iron and bronze, and causes a horrible smell to fill the air. Dogs who taste the blood become mad, and their bite becomes poisonous as in rabies. The Dead Sea, thick with salt, cannot be drawn asunder except by a thread soaked in the poisonous fluid of the menstruous blood. A thread from an infected dress is sufficient. Linen, touched by the woman while boiling and washing it in water, turns black. So magical is the power of women during their monthly periods that they say that hailstorms and whirlwinds are driven away if menstrual fluid is exposed to the flashes of lightning” from **Pliny the Elder, *Natural History*, book 28, ch. 23, 78-80; book 7, ch. 65.**



PHYSIOLOGICAL REVIEWS

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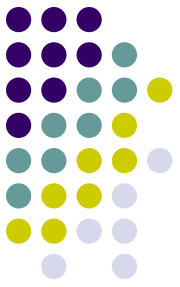
OESTRUS, OVULATION AND MENSTRUATION

GEORGE W. CORNER

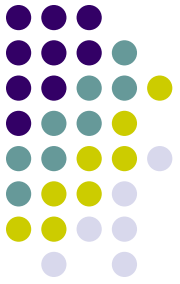
Department of Anatomy, The Johns Hopkins University

A long series of traditional and scientific writings testifies to the mystification produced by the menstrual periodicity of the human female. We find the Mosaic lawgivers already concerned to regulate the hygiene of menstruation; thousands of years later an Arrhenius is still puzzling over the relation between the cycles of menstruation and those of the moon. In recent years, however, there seems to have been a genuine advance toward a biological explanation of the reproductive cycle in mammals, and we may hope that the general enlightenment is finally extending to the undoubtedly peculiar problems of our own species.

Markee JE. Menstruation in intraocular transplants in the rhesus monkey. Contributions to Embryology of the Carnegie Institution. 1940;177:211–308.



- Endometrial intraocular implants
- Direct visualisation of spiral arterioles
- Process of bleeding
- Vasoconstriction/ vasodilatation
- Haemostasis
- ‘During menstruation, these arterioles undergo profound vasoconstriction with bleeding occurring as they dilate’



(FROM THE PATHOLOGICAL DEPARTMENT OF THE KAROLINSKA SJUKHuset, STOCKHOLM. HEAD: PROFESSOR F. HENSCHEN, AND THE ANATOMICAL DEPARTMENT OF THE KAROLINSKA INSTITUTE. HEAD: PROFESSOR TURE PETRÉN.)

The Blood Vessels of The Human Endometrium.

A histological study by means of injection and blood corpuscle colouration.¹

(Received for publication sept. 5th 1945).

By

JÖRGEN BREMS DALGAARD.

Aarhus.

Review of the Literature.

THE BLOOD VESSELS OF THE HUMAN ENDOMETRIUM

343

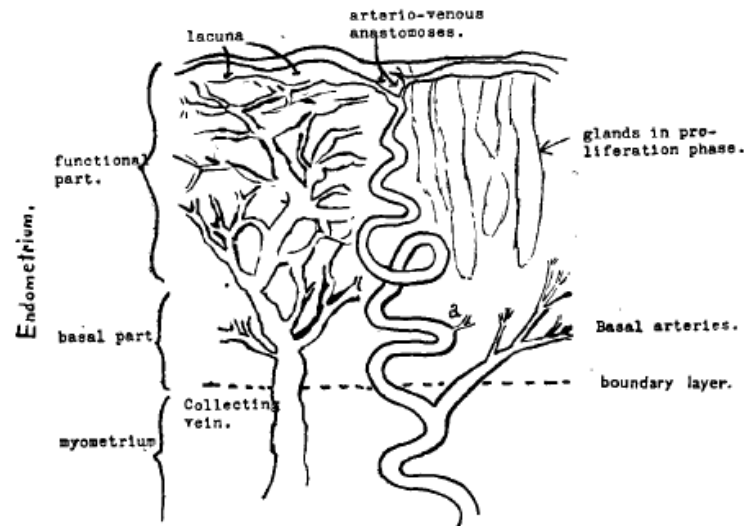


Fig. 1. Highly Schematic View of the Endometrial Blood Vessel System.

Prior to the 20th century, European and American women had fewer periods

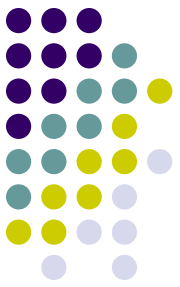


- They started menstruating later, frequently in the mid to late teens, and stopped earlier, if they lived long enough to experience menopause, thus creating a shorter time for menstruation
- married earlier, legitimizing the production of children, which reduced menstruation
- had more children, and used less contraception, stopping menstruation for long periods
- breast fed their children longer (and more often), which usually stopped menstruation
- were more likely to be under- and malnourished or sick, or any combination thereof, which can stop menstruation
- died earlier - stopping it dead

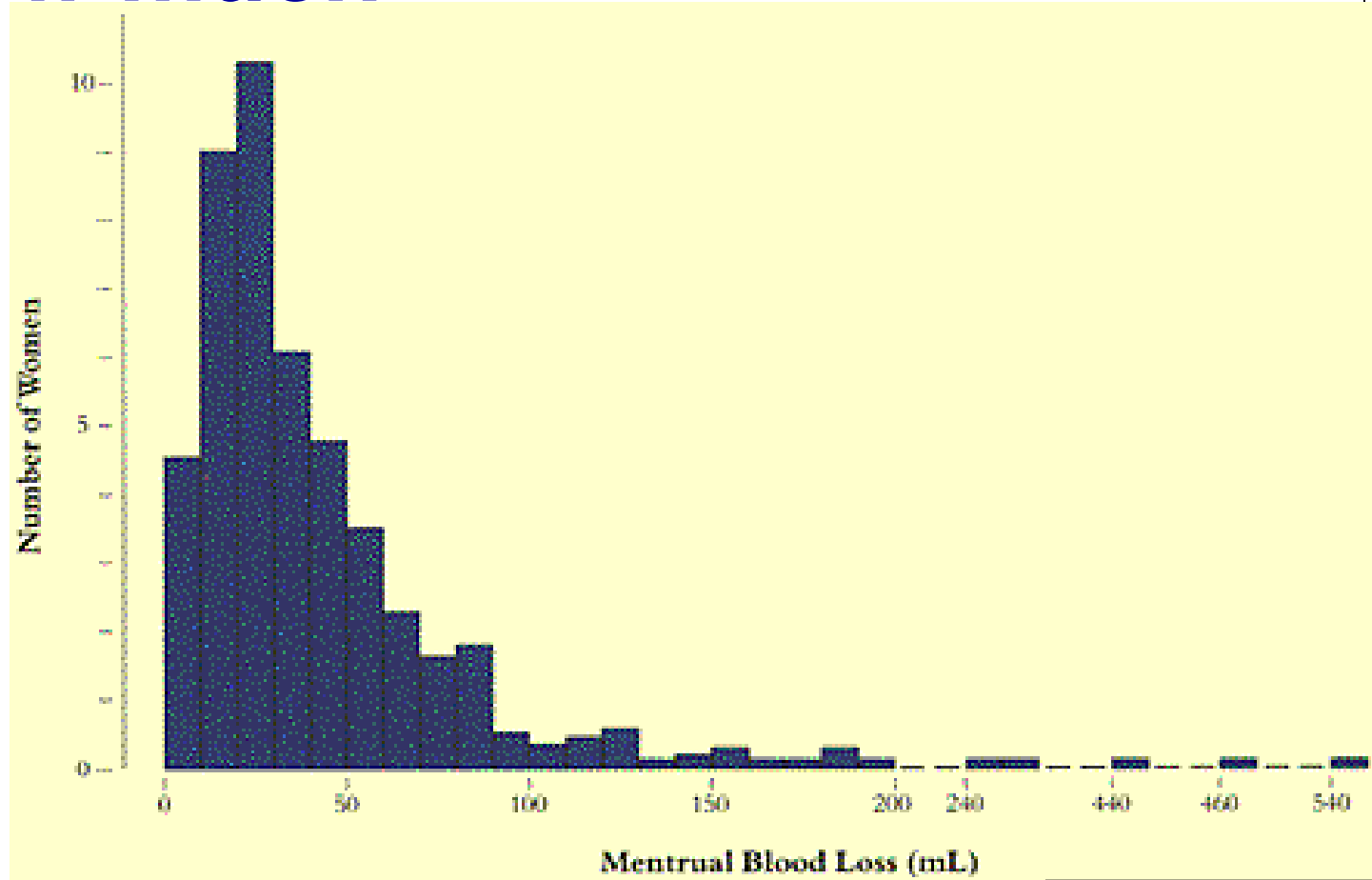
Menstruation from menarche to menopause



- 400
- average duration 5-6 days
- time spent bleeding 5-7 years
- average volume blood lost 14 litres
- Monthly bleeding will continue with sequential HRT



How much



Hallberg et al 1996

Mean blood loss: Sweden 43 ml/ China 56 ml

Blood loss and age: Hallberg et al 1966



Age

Median blood loss (mls)

15

28.4

23

30.6

30

30.9

40

30.8

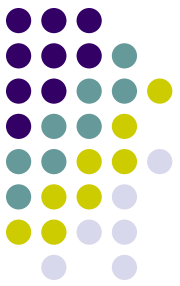
45

29.5

How long



Study	Country	Mean duration (days)
WHO 1981	Mexico	4.0
	India	4.4
	Egypt	4.4
	Yugoslavia	4.8
	UK	5.3
Belsey et al 1988	Mexico	4.0
	Europe	5.9
WHO 1986	Hongkong	6.0



How often: menstrual cycle length

Treloar et al 1967, Vollman et al 1977

	Median length (days)	Range
Postmenarche year 1	29	18-80
Age 20	28	20-40
Age 30	28	23-40
Age 40	26	23-33
Premenopause 5 years	26	18-39
Premenopause 2 years	27	16-80
Premenopause 1 year	28	15- infinity

Models to understand uterine bleeding



- Where and when is not enough
- Need to functional assays
- What does an agent do?
- In vitro models
- In vivo models
- Zhang L, Rees MCP and Bicknell R (1995) The isolation and long term culture of normal human endometrial epithelium and stroma. Expression of mRNA's for angiogenic polypeptides basally and on oestrogen challenge. J Cell Science. 108:323-331
- Nikitenko LL, MacKenzie IZ, Rees MC, Bicknell R.(2000) Adrenomedullin is an autocrine regulator of endothelial growth in human endometrium. Mol Hum Reprod. 6: 811-819.

Endometrial angiogenesis



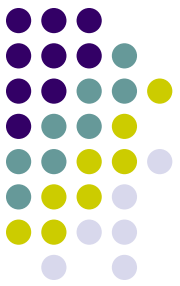
• Known Angiogenic Growth Factors

Adrenomedullin

- Angiogenin
- Angiopoietin-1
- Del-1
- Fibroblast growth factors: acidic (aFGF) and basic (bFGF)
- Follistatin
- Granulocyte colony-stimulating factor (G-CSF)
- Hepatocyte growth factor (HGF) /scatter factor (SF)
- Interleukin-8 (IL-8)
- Leptin
- Midkine
- Placental growth factor
- Platelet-derived endothelial cell growth factor (PD-ECGF)
- Platelet-derived growth factor-BB (PDGF-BB)
- Pleiotrophin (PTN)
- Progranulin
- Proliferin
- Transforming growth factor-alpha (TGF-alpha)
- Transforming growth factor-beta (TGF-beta)
- Tumor necrosis factor-alpha (TNF-alpha)
- Vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF)

• Known Angiogenesis Inhibitors

-
- Angioarrestin
- Angiostatin (plasminogen fragment)
- Antiangiogenic antithrombin III
- Cartilage-derived inhibitor (CDI)
- CD59 complement fragment
- Endostatin (collagen XVIII fragment)
- Fibronectin fragment
- Gro-beta
- Heparinases
- Heparin hexasaccharide fragment
- Human chorionic gonadotropin (hCG)
- Interferon alpha/beta/gamma
- Interferon inducible protein (IP-10)
- Interleukin-12
- Kringle 5 (plasminogen fragment)
- Metalloproteinase inhibitors (TIMPs)
- 2-Methoxyestradiol
- Placental ribonuclease inhibitor
- Plasminogen activator inhibitor
- Platelet factor-4 (PF4)
- Prolactin 16kD fragment
- Proliferin-related protein (PRP)
- Retinoids
- Tetrahydrocortisol-S
- Thrombospondin-1 (TSP-1)
- Transforming growth factor-beta (TGF-b)
- Vasculostatin
- Vasostatin (calreticulin fragment)

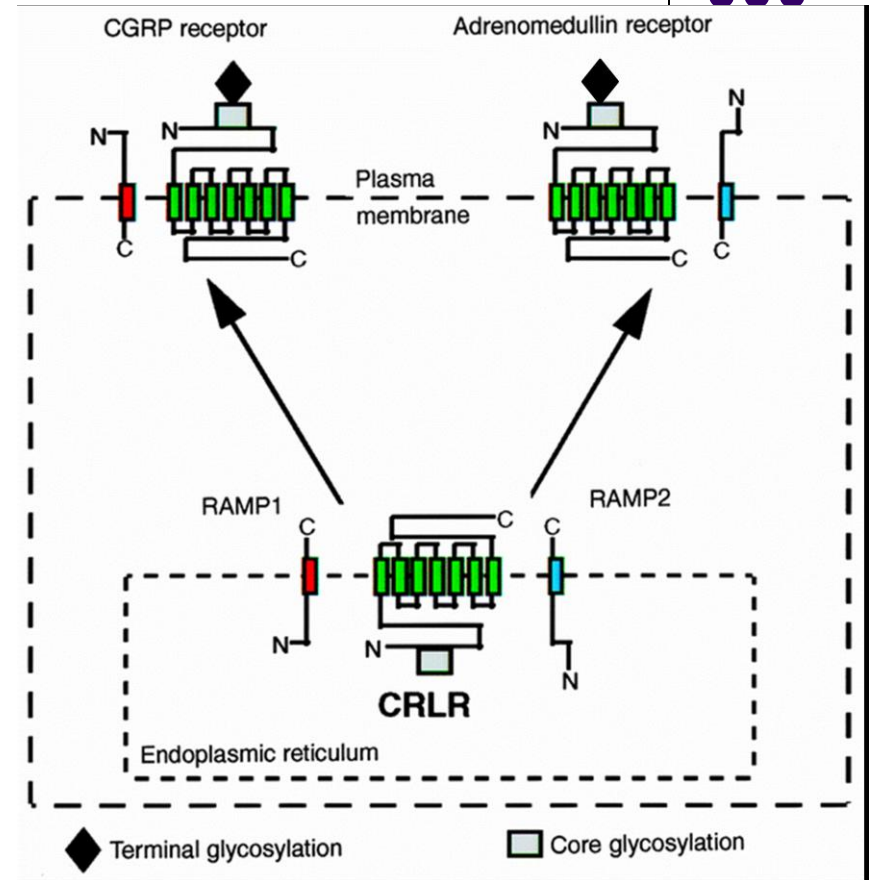


Adrenomedullin

- 52-amino acid peptide originally isolated from a human pheochromocytoma
- **Properties:**
 - 1) it is a known **growth factor**
 - 2) it has **angiogenic activity** both *in vitro* and *in vivo*
 - 3) it **inhibits apoptosis** in isolated endometrial carcinoma cells and endothelium
 - 4) it causes **vasodilatation**

AM Receptors

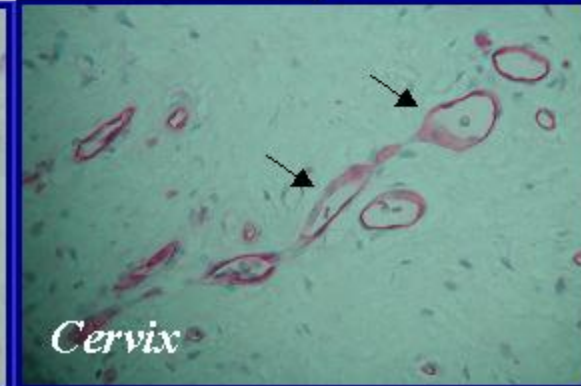
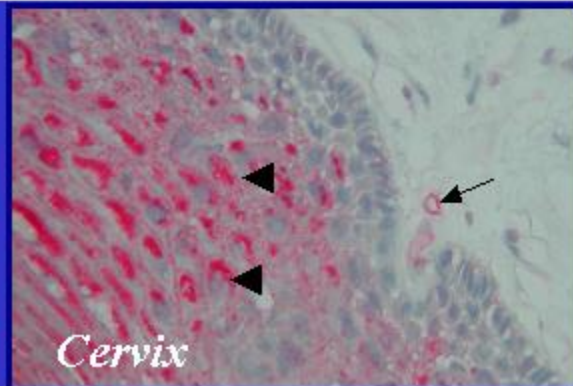
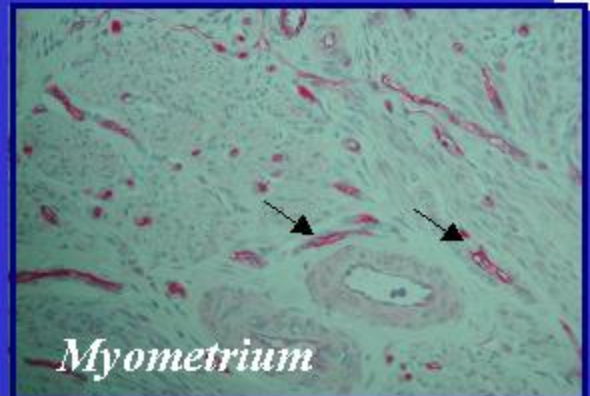
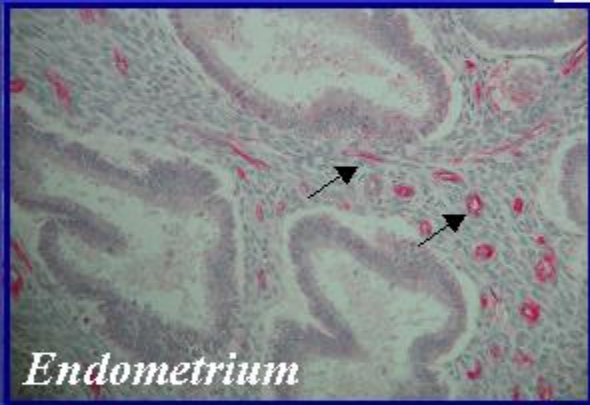
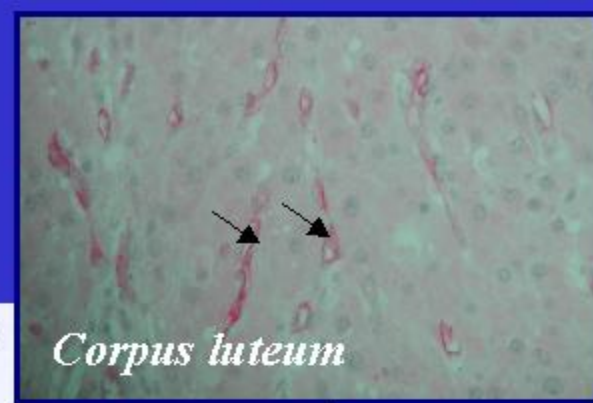
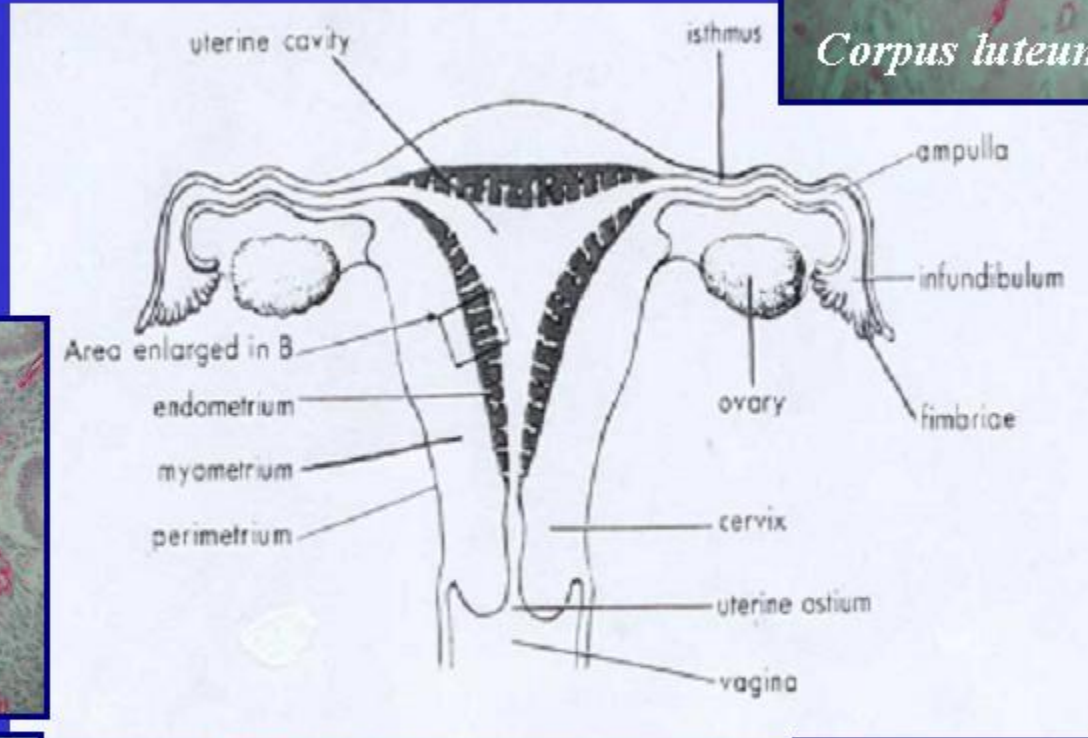
- G-protein coupled receptor (GPCR) Calcitonin receptor-like receptor (CRLR or CL)
- Receptor activity modifying proteins (RAMPs)
- CL expression is regulated by hypoxia
- RAMP expression not altered by hypoxia



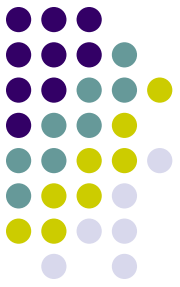
L.M. McLatchie et al. Nature Vol. 393 May 1998

Modified according to the Hilaiet et al. J Biol Chem. 2001; 276(31), 29575-81.

Localisation of hCL in vivo



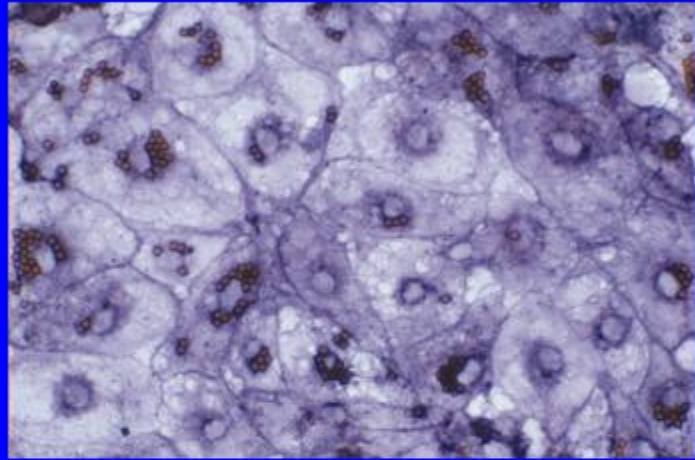
Chick chorioallantoic membrane (CAM) assay



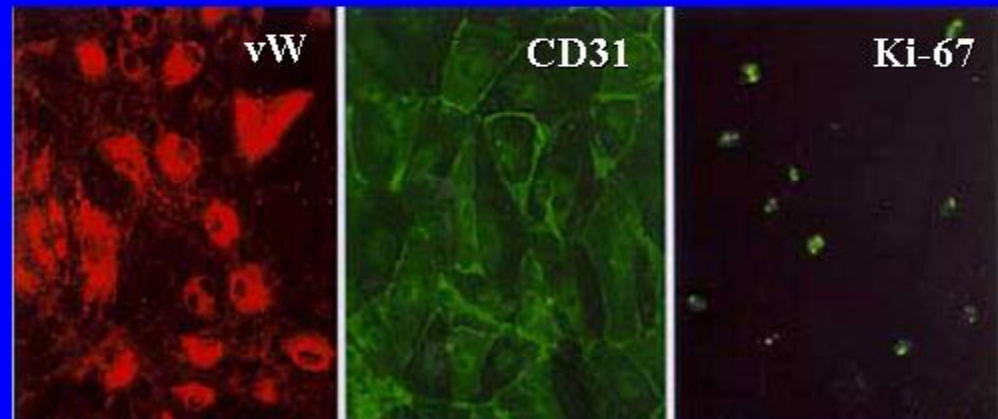
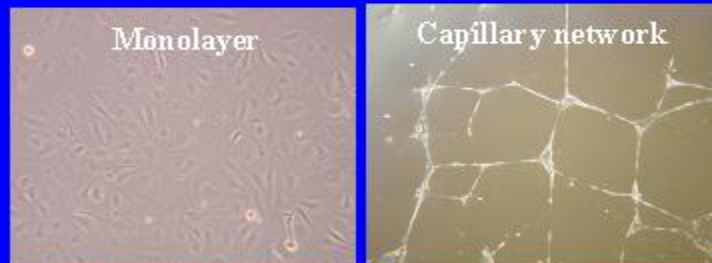
- Agents are placed on the CAM through a window made in the eggshell. This causes a typical radial rearrangement of vessels towards, and a clear increase of vessels around the graft within four days after implantation. Blood vessels entering the graft are counted under a stereomicroscope.



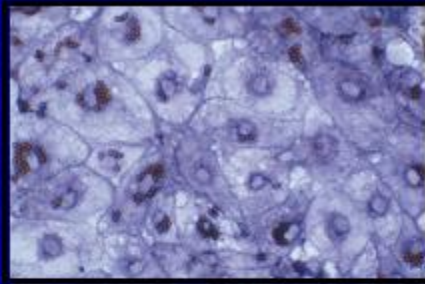
Isolation and characterization of human microvascular endothelial cells.



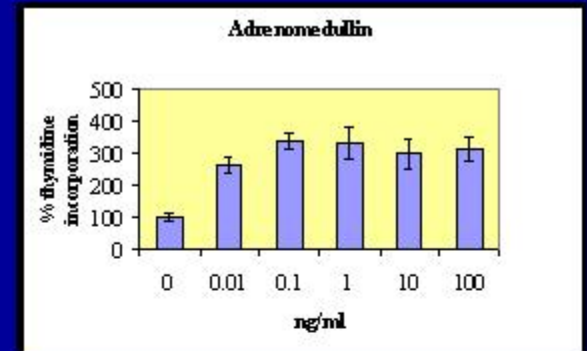
Positive selection of
decidual, endometrial and myometrial
microvascular endothelial cells
with Dynabeads-*UEA*



Primary human microvascular EC

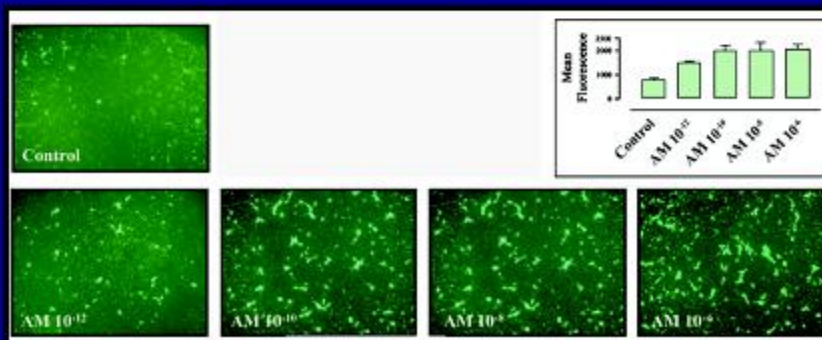


Proliferation



Studies of the role of adrenomedullin in endothelial cell biology

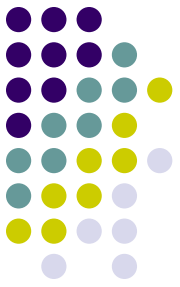
Migration



Tube formation

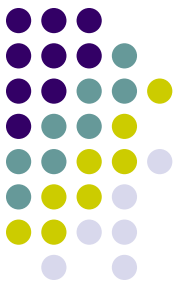


Withdrawal bleeds with sequential HRT



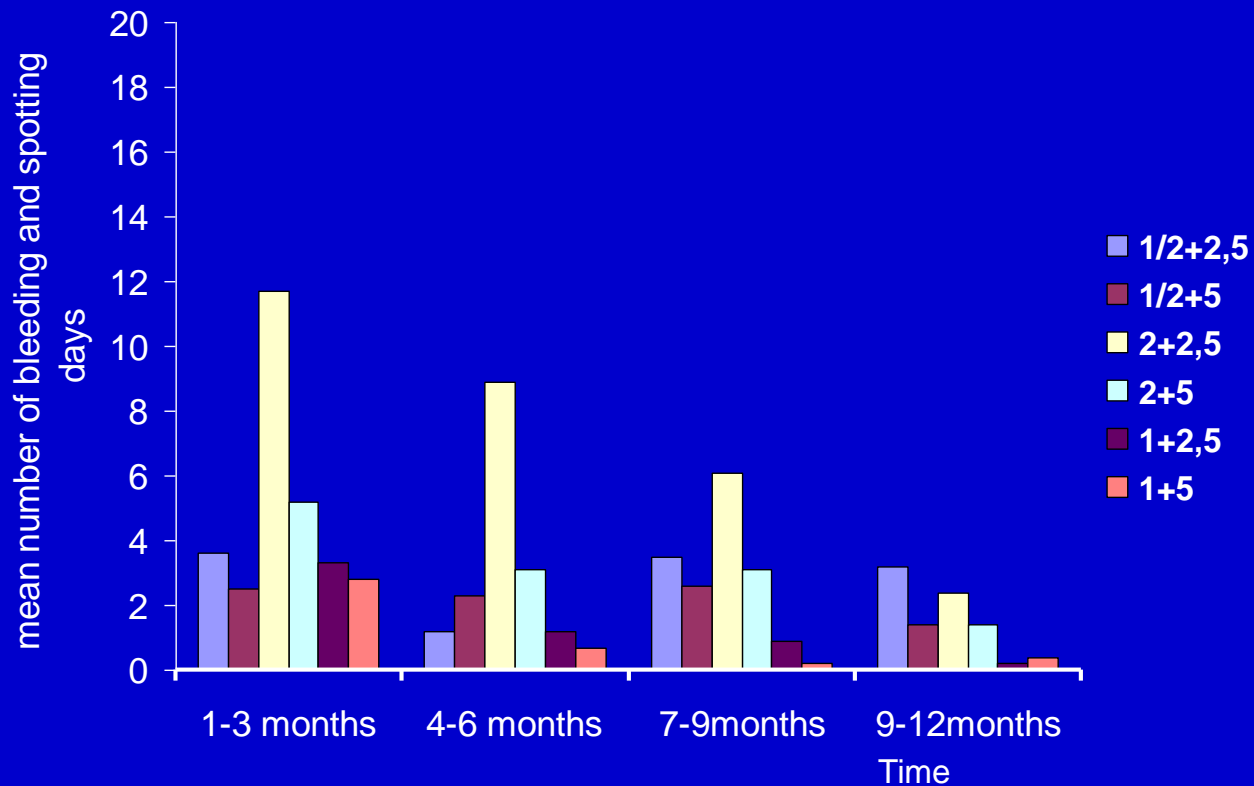
- No greater than premenopausal periods
- Rees and Barlow, Quantitation of hormone replacement induced withdrawal bleeds. *Br J Obstet Gynaecol* 98 (1991), pp. 106–07.
- Sporrong, Rybo, Mattson, Vilbergson and N. Crona, An objective and subjective assessment of uterine blood loss in postmenopausal women on hormone replacement therapy. *Br J Obstet Gynaecol* 99 (1992), pp. 399–401.

Tibolone and low-dose continuous combined hormone treatment: vaginal bleeding pattern, efficacy and tolerability.
Hammar et al ; TOTAL Study Investigators Group.
BJOG. 2007;114:1522-9.



- Multicentre study executed in 32 centres in 7 European countries.
- Five hundred and seventy-two healthy symptomatic postmenopausal women, aged 45-65 years.
- Participants were randomised to receive 2.5 mg tibolone or 1 mg 17beta estradiol plus 0.5 mg norethisterone acetate (E2/NETA) daily for 48 weeks.
- The incidence of bleeding was significantly lower in the tibolone group during the first 3 months of treatment (18.3 versus 33.1%; $P < 0.001$) when compared with the E2/NETA group. This effect on the bleeding pattern was sustained throughout the study, although reaching statistical significance again only in 7-9 months of treatment (11 versus 19%; $P < 0.05$).

Mean number of cumulative
bleeding and spotting days
with oestradiol valerate and MPA Heikkinen et al Am J
Obstet Gynecol 2000



Levonorgestrel intrauterine system (LNG-IUS) with conjugated oral equine estrogen: a successful regimen for HRT in perimenopausal women.

Hampton et al Hum Reprod. 2005;20:2653-60.

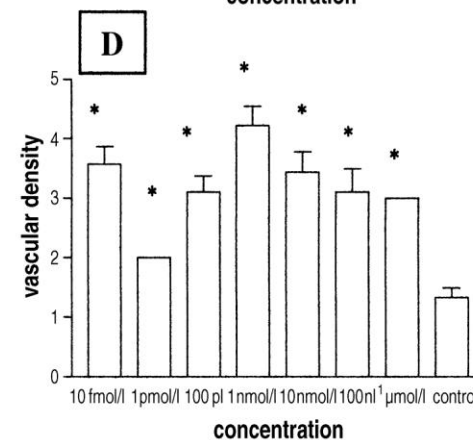
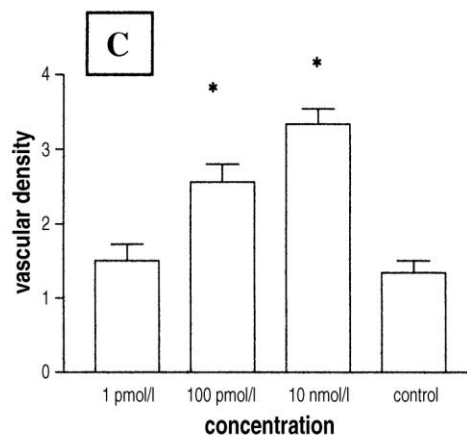
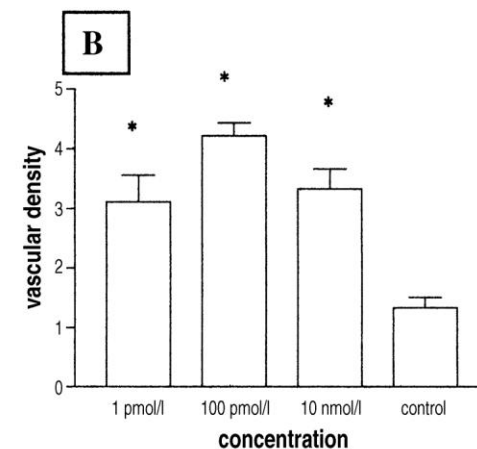
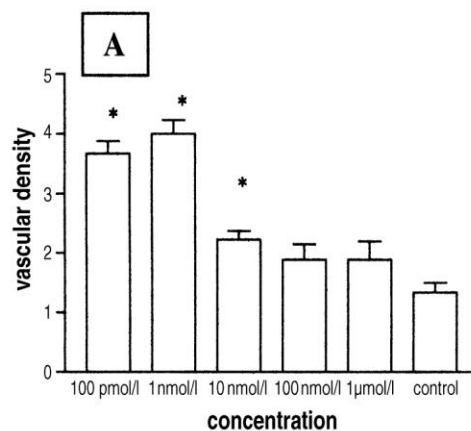


- 5 year study of the levonorgestrel-releasing intrauterine system (LNG-IUS) in 82 women received oral conjugated equine estrogen 1.25 mg daily.
- The proportion of amenorrhoeic women increased from 54.4% at 12 cycles to 92.7% at the end of the study.
- The continuation rate per 100 women at 60 cycles was 79.84 (95% CI 71.0-88.6).

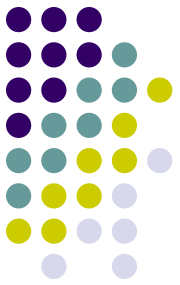
In-vivo angiogenesis and progestogens

Hague, S. et al. Hum. Reprod. 2002 17:786-793

- In the mouse sponge assay, norethisterone and medroxyprogesterone acetate stimulated angiogenesis at all doses, but was dose-dependent for levonorgestrel and nomegestrol. Levonorgestrel stimulated angiogenesis in the dose range 100 pmol/l to 10 nmol/l, but not at higher doses. In contrast, nomegestrol acetate stimulated angiogenesis at high, but not low, doses.



Assessment of microvascular density in the mouse subcutaneous sponge assay, after immunostaining for von Willebrand factor. (A) Levonorgestrel; (B) medroxyprogesterone acetate; (C) nomegestrol acetate; (D) norethisterone. Values are mean \pm SE (n = 3).*, significant increase in vascular density when compared with control.



- Sir Francis Darwin (1848 - 1925),
Eugenics Review, April 1914
- The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.
- Leonid Nikitenko
- Martin Oehler
- Stephen Hague
- Lyna Zhang
- Yuan Zhao
- Janet Brockie
- Caroline Naish
- Roy Bicknell