The menopause and its management, a revisit.

Mechanisms of irregular bleeding with hormone therapies

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"Mechanisms of irregular bleeding with hormone therapies "

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- MRC
- NIH CHD (R01- HD - 43209-03)
- TAP Pharmaceuticals, USA
- Schering

Overview

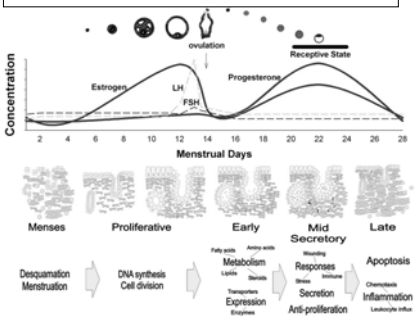
"Mechanisms of irregular bleeding with hormone therapies "

- **Clinical problem: unscheduled endometrial bleeding**
- **Normal endometrial cycle**
- **Endometrial steroid receptor expression patterns**
- **Mechanisms involved in normal menstruation**
- **Local mediators implicated in endometrial bleeding**
- **Bleeding with progestogen-only hormone therapies**
- **Bleeding with hormone replacement therapy**

"Mechanisms of irregular bleeding with hormone therapies " Clinical Problem

- **HRT used by peri- and postmenopausal women for relief of menopausal symptoms**
- **Therapeutic benefit from oestrogen replacement; progestogen added for endometrial protection**
- **Many women use a continuous combined preparation to avoid withdrawal bleeding**
- **Clinical problem: unscheduled endometrial bleeding in up to 60% of HRT users – thus discontinuation of therapy in 1 in 3 users**
  (al-Azzawi & Habiba 1994 *BJOG* 101:661-2; Limouzin-Lamonthe 1996 *Eur J Obstet Gynecol Reprod Biol* 64:S21-24)
- **30% of cyclic HRT users and near half of continuous combined users make a minimum of 1 visit to gynaecologist for problematic bleeding –in majority no pathology found. Invasive and expensive investigations to exclude malignancy**
  (Ettinger et al 1998 *Fertil Steril* 69: 865-9; Elliot et al 2003 *Acta Obstet Gynecol Scand* 82: 112-119; Hickey et al 2005 *J Clin Endocrinol Metab* 90:5528-35)

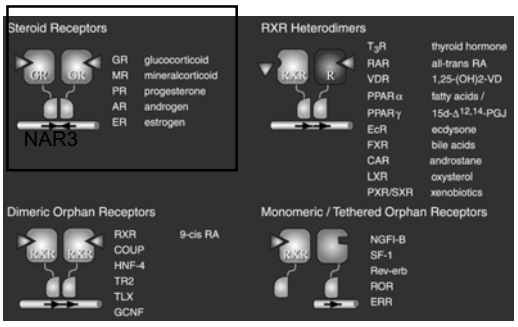
The human menstrual cycle



Simmen & Simmen 2006; Endocrinology 147: 1094-1096

Talbi *et al* 2006; Endocrinology 147: 1097-1121.

Steroid receptor superfamily



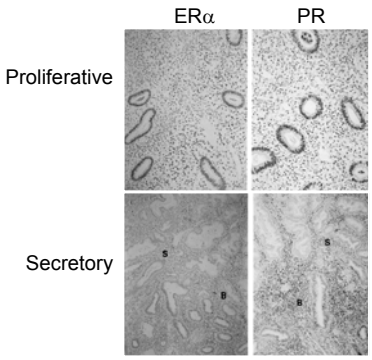
Olefsky 2001*; JBC 276:*

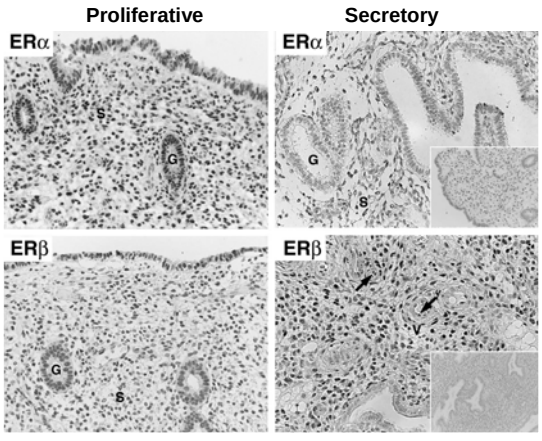
Overview of steroid receptor expression in endometrium

Protein expression	Proliferative		Secretory		Decidua		uNK cells
	Glands	Stroma	Glands	Stroma	Glands	Stroma	
PR	+	+	–	+	–	+	–
ERα	+	+	+/–	+/–	–	+/–	–
ERβ1	+	+	+	+	+	+	+
ERβcx/β2	+	+	+/–	+	+	+	–
GR	–	+	–	+	+	+	+

Henderson TA, Saunders PT, Moffett-King A, Groome NP, Critchley HO 2003
Steroid receptor expression in uterine natural killer cells. J Clin Endocrinol Metab 88:440-449

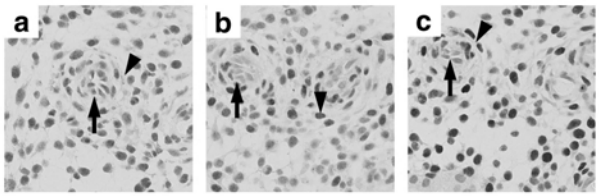
Normal Cycle





Uterine endothelial cells - ERß positive

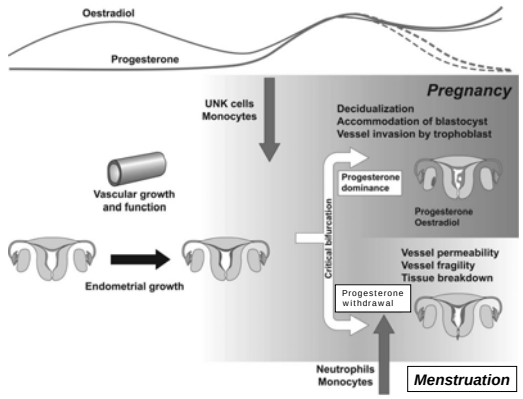
ERβ ERα PR



Critchley et al 2001 *JCEM* 86: 1370

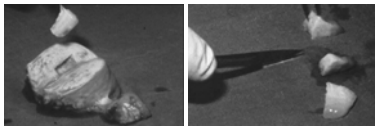
Normal endometrial cycle

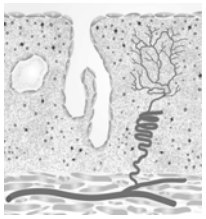
- **Unopposed oestrogen** (**E)** exposure promotes regeneration and proliferation post-menses
- **E** induces expression of ER & PR
- Period of **unopposed E** exposure essential for up-regulation of PR. The endometrium responds to **progesterone (P)** in luteal phase - differentiation
- **P** essential for establishment of pregnancy **following** a period of **unopposed E** exposure.

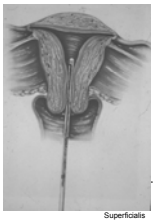


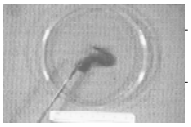
Jabbour, Kelly, Fraser, Critchley 2006 *Endocrine Reviews* 27:17- 46

Local endometrial events are spatially and temporally regulated – sampling techniques









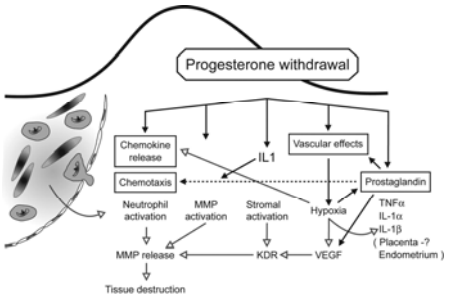
Menstruation: an inflammatory event

- Many lines of evidence underpin menstruation as an inflammatory event with tight temporal and spatial regulation at molecular and cellular levels.
- The functional layer of the human endometrium undergoes serial degeneration and renewal each menstrual cycle.
- Withdrawal of progesterone (P) due to luteal regression initiates the breakdown of the upper functional zone at menses.
- Novel injury-repair mechanisms:
- Progesterone - withdrawal and modulation of local steroid signalling
  - up-regulation of local inflammatory mediators
  -up-regulation of factors orchestrating ECM remodelling and vasculogenesis

Critchley et al 1999; Milne et al 1999; J Clin Endocrinol Metab. 84: 240 & 2563
Nayak et al 2000; J Clin Endocrinol Metab 85: 3442-52
Brenner et al 2002; Ann NY Acad Sci 955: 60-74;
Hapangama et al 2002; J Clin Endocrinol Metab 87: 5229-34

Progesterone withdrawal activates many pathways; prominent among these are those releasing vasoactive agents

Jabbour, Kelly, Fraser, Critchley *Endocrine Reviews* 2006;27:17-46



American Journal of Obstetrics and Gynecology (2006) 195, 406.e1–406.e16

ELSEVIER

American Journal of Obstetrics & Gynecology
www.ajog.org

Gene expression profiling of mid to late secretory phase endometrial biopsies from women with menstrual complaint

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Gene expression profiling of Mid secretory and Late secretory phases of Menstrual cycle

10 women

5 mid secretory phase pipelle/ hysterectomy samples

5 Late secretory phase pipelle / hysterectomy samples

Expression in each independent sample analysed using HG-U133A arrays

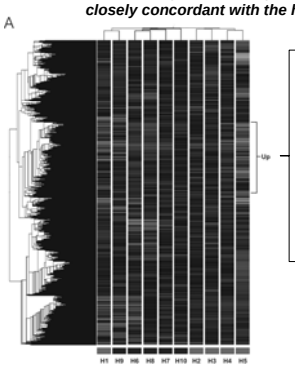
↓

Allow analysis of around 22,000 transcripts

Explorative analysis

Hierarchical clustering of variable genes (CV>0.2) results in a segregation closely concordant with the histological classification

A



Several well-known local mediators of endometrial function are up-regulated in the premenstrual tissue Including:

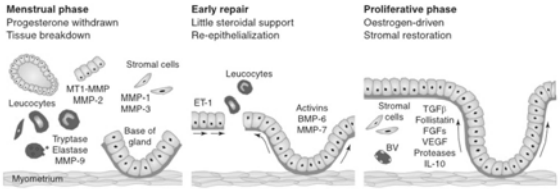
-Decidualisation (IGFBP-1, prolactin, prolactin receptor)

- Matrix metalloproteinase family (MMP-10, MMP-7, TIMP-2,-3)

-Endometrial bleeding associated factor (ebaf)

-Endothelin (ET-1) and

- ETB receptor 2,3,4



Salamonsen *Reproduction* 2003 125: 301 - 311

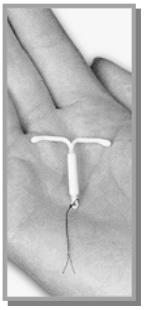
Heavy Menstrual Bleeding (HMB)

Local uterine causes	Iatrogenic causes	Systemic causes	Endometrial causes
Leiomyoma	Anticoagulants	Coagulation disorders	Altered synthesis of uterine vasodilatory prostanoids
Polyp	Copper intrauterine device	Hypothyroidism	Reduced endothelin expression
Infection		Chronic liver disease	Increased fibrinolysis
Carcinoma		Chronic cardiac or renal disease	Perturbed endometrial angiogenesis
Adenomyosis			Perturbed endometrial regeneration
Pelvic A-V malformation			Overproduction of nitrogen oxide

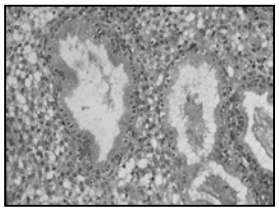
Hormone therapies acting on the uterus

- Combined oral contraceptive pill (COCP)
- Exogenous systemic progestogens: POP; subdermal P implants; Depo provera
- Intrauterine delivery of LNG (LNG-IUS)
- Hormone replacement

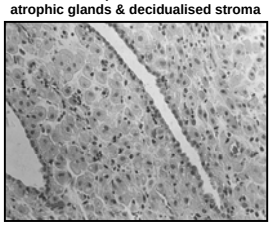
The levonorgestrel-releasing
(LNG-IUS) intrauterine system
Endometrial response to LNG



Normal secretory phase endometrium



Endometrium post LNG IUS insertion atrophic glands & decidualised stroma

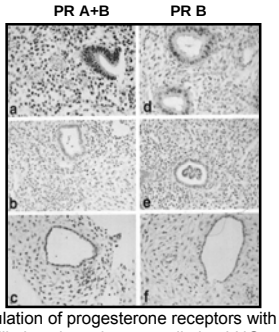


- down regulation of endometrial sex steroid receptors
- changes in blood vessel integrity

Endometrial response to intrauterine LNG

- Histology
  - Endometrial atrophy
  - Extensive decidualization
  - Altered spiral artery formation
  - Superficial thin-walled dilated blood vessels
- Immunohistochemistry
  - Down-regulation of estrogen receptor, progesterone receptor and androgen receptor
  - Increased leukocyte infiltration (uNK, macrophages)
- Local factors
  - Cytokine and prostaglandin up-regulation
  - Altered angiogenesis (VEGF ↑)
  - MMP up-regulation TF ↑, IGFBP-1 ↑
- Intracrinology
  - 17βHSD-2 up-regulated
  - E2 ↓, E1 ↑

Progesterone Receptor (PR) immunostaining



Down regulation of progesterone receptors with LNG IUS PR A likely to be subtype mediating LNG action

Critchley *et al*. 1998

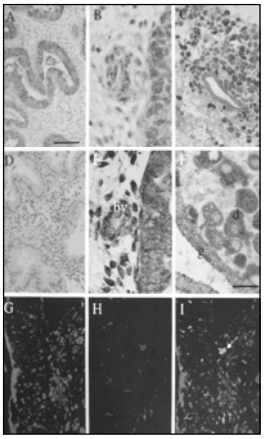
Disturbed endometrial bleeding patterns and perturbed morphology

- May be due to changes in vessel integrity
- Angiogenesis is influenced by both endocrine and paracrine factors
- Vascular endothelial growth factor (VEGF) plays a major role in angiogenesis
- Hormone manipulation may perturb angiogenesis

Lebovic et al 2000 *Hum Rep* 15 (Suppl 3) 67 -77

Vascular Endothelial Growth Factor- VEGF

- Potent angiogenic and mitogenic factor present in endometrium (*Smith 1998*)
- Stimulates MMP synthesis *(Ahmed et al 1997)*
- Binds to its receptors VEGFR-1(ftl-1) and VEGFR-2 (KDR)predominantly expressed in endothelial cells (*Skobe et al 1997*)
- VEGF and KDR present in decidualized stroma cells of endometrium just prior to menses *(Nayak et al 2000)*



C, F: VEGF immunostaining in LNG exposed endometrium

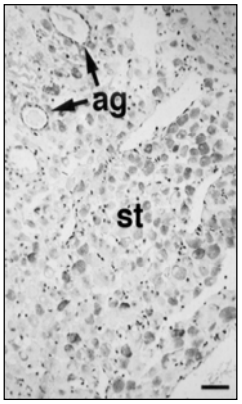
G, H, I: serial sections stained for VEGF and CD68 (macrophages)

Charnock-Jones et al.
Hum Reprod 15: (Supp. 3) 85-95

Matrix metalloproteinases (MMPs) in endometrium

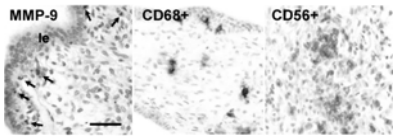
- Evidence role for MMPs in menstrual bleeding
- MMPs are enzymes responsible for breakdown/remodelling of extracellular matrix
- Focal pattern of expression suggests local rather than hormonal regulation
- Leukocytes in endometrium may release MMPs
- Interactions between leukocytes and stromal and epithelial cells induce and activate MMPs

MMP-9 (LNG-IUS) = up-regulated

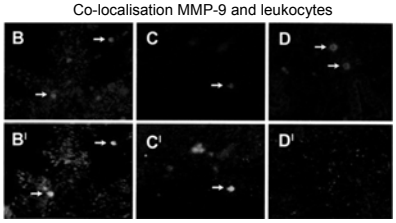


Skinner et al. 1999
Hum Reprod 14: 793-799

MMP-9 immunoreactivity in DMPA- users endometrium



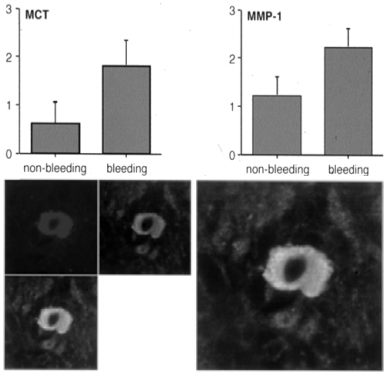
Co-localisation MMP-9 and leukocytes



neutrophils CD3 + Tcells mast cells

Adapted from Vincent et al 2002 *Hum Reprod* 17: 1189

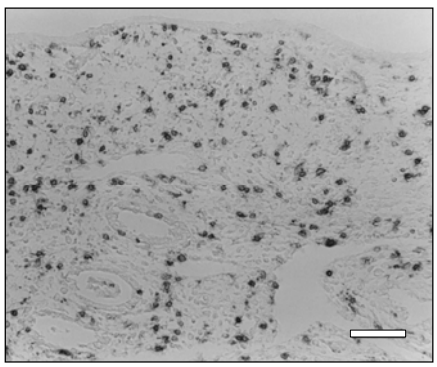
Mast cells

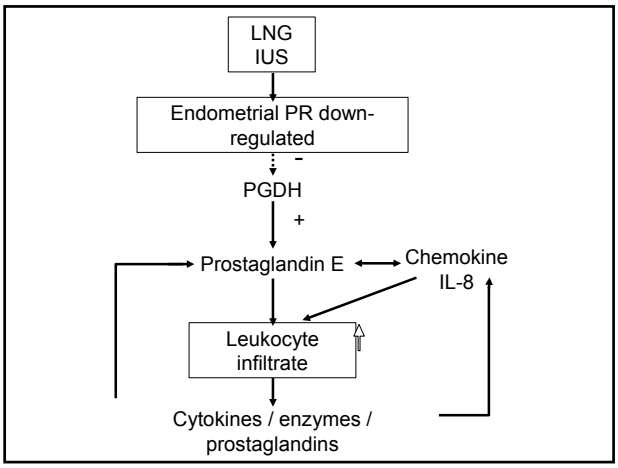


MMP-1

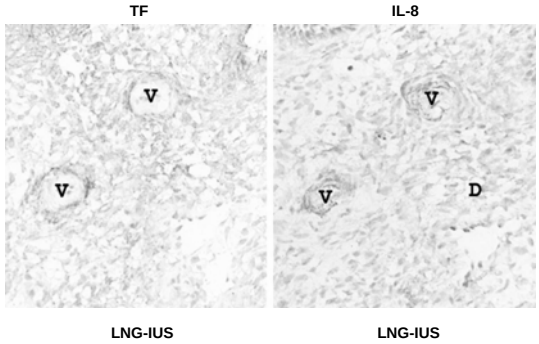
Milne et al 1999; *J Clin Endocrinol Metab* 84:2563

Leukocytes in LNG-treated endometrium





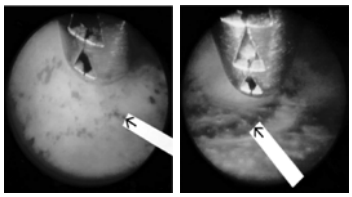
TF and IL-8 Immunoreactivity



Lockwood *et al.* 2004 *J Clin Endocrinol Metab* 89:1467

Progestin - only contraception (Norplant) and B-T-B

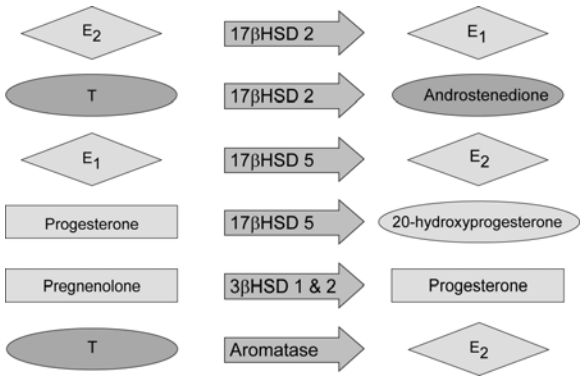
- enlarged, thin-walled vessels
- vascular fragility ↑
- trend toward ↓ endometrial perfusion



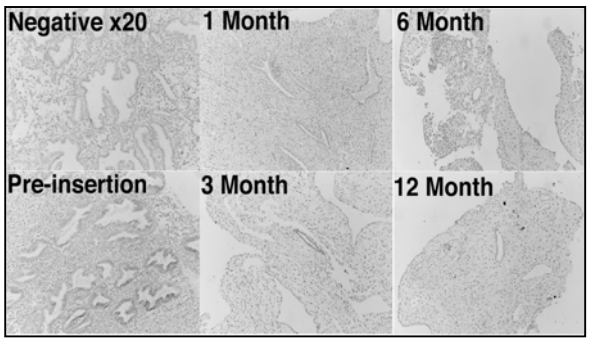
Hickey *et al* 2000
Hum Reprod 15:1509-14

Endometrial intracrinology

Ligand availability dependent upon local cellular enzymes.

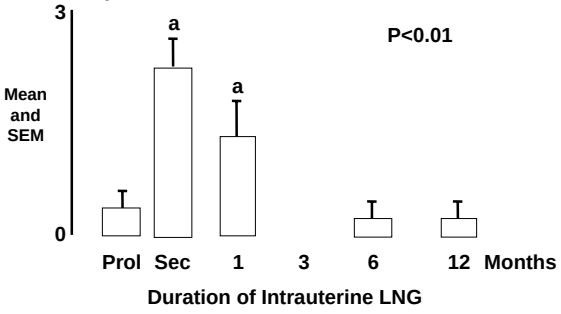


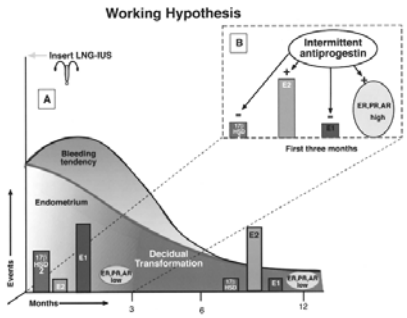
17β HSD2 immunostaining



Burton et al 2003
Hum Reprod 18:2610

17β HSD2 immunostaining enzyme expression inhibited with LNG





Collaborative Research Program (NIH);
Edinburgh/Oregon National Primate Research Center

Mechanisms of bleeding on menopausal hormone therapy

(Reviewed in Hickey, *Menopause Int.* 2007)

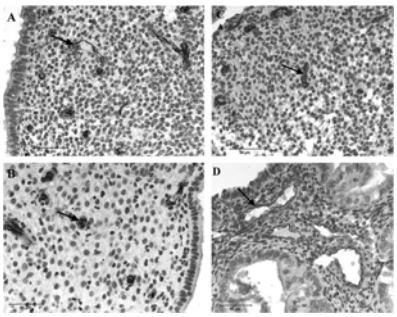
- Mechanisms poorly understood
- No correlation with histology or dose of hormone therapy **(Thomas, Hickey, Fraser. *Hum Reprod* 2000)**
- Endometrial bleeding involves breakdown of endometrial vessels and overlying epithelium **(Hickey, *Menopause Int.* 2007)**
- Endometrial vascular breakdown is locally regulated **(Smith, *Hum Reprod* 2000)**
- Endometrial effect of continuous combined hormone replacement is largely progestogenic **(Wells, Sturdee, Barlow et al. *BMJ* 2002)**
- Some of the mechanisms implicated in unscheduled bleeding with HRT may resemble those involved with irregular bleeding experienced by women using progestin-only contraception

Potential mechanisms underlying bleeding in users of combined HRT

(Summarised in Hickey, Menopause Int. 2007)

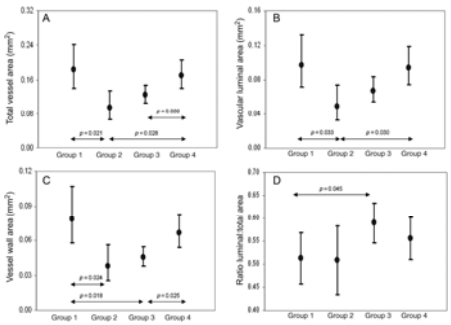
- Alterations in endometrial vasculature - changes in vessel size & stromal expression of factors regulating vessel growth and integrity **(Hickey et al 2008; *Hum Reprod* 23:912-8)**
- Disturbances in expression of MMPs and their tissue inhibitors – TIMPs **(Hickey et al 2006; *J Clin Endocrinol Metab* 91:3189-98; Hickey et al 2001; *Fertil Steril* 75:288-96)**
- Increased endometrial stromal leukocytes - CD56+uterine NK cells increased during bleeding episodes **(Hickey et al 2005; *J Clin Endocrinol Metab* 90:5528-35)**

Representative photographs of vessels in biopsies taken from (A) subject not using HT; (B) subjects taking HT for >3m with no irregular bleeding: (C) subject taking HT for >3m with irregular bleeding and (D) subject taking HT for >3m with irregular bleeding at the time of biopsy



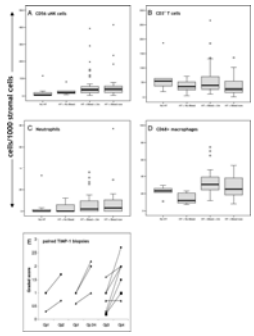
Hickey, M. *et al. Hum. Reprod.* 2008 23:912-918

Vessels area statistics stratified by HRT exposure and bleeding patterns in HRT users (estimated means and their 95% confidence intervals)



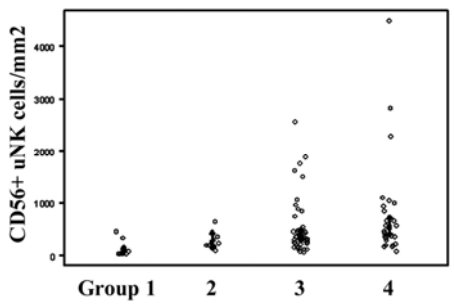
Hickey, M. et al. *Hum. Reprod.* 2008 23:912-918

Relative cell numbers per 1000 stromal cells of CD56+ uNK cells (A), CD3+ T cells (B), polymorphic neutrophils (C), and CD68+ macrophages (D)



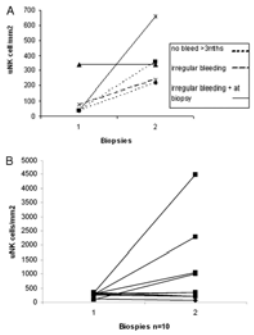
Hickey, M. et al. *J Clin Endocrinol Metab* 2006;91:3189-3198

Irregular bleeding with HRT
Scatter graph of CD56+ cell density/mm2 within groups



Hickey, M. et al. *J Clin Endocrinol Metab* 2005;90:5528-5535

Comparing uNK cell densities in paired biopsies before and after more than 3 months HRT (n = 5)



Hickey, M. et al. *J Clin Endocrinol Metab* 2005; 90:5528-5535

Mechanisms of bleeding on menopausal hormone therapy

(Vani et al 2008; *J Fam Plann Reprod Health Care* 34:2-34)

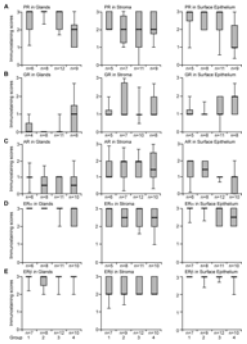
- Mechanisms of HRT-related bleeding likely mediated through endometrial steroid receptors.
- Steroid receptor expression studied in HRT-exposed endometrium in relation to disturbances of bleeding patterns.
- Prospective observational study; 21 post-menopausal women examined.
- IHC performed for PR, ERα, ERβ, AR and GR.

Steroid receptor expression in postmenopausal endometrium

Group	Women (*n*)	Group definitions			Biopsies (*n*)
		Hormone replacement therapy treatment >3 months	History of unscheduled bleeding	Bleeding at time of endometrial biopsy	
1	7	No	No	No	7
2	8	Yes	No	No	9
3	9	Yes	Yes	No	12
4	9	Yes	Yes	Yes	10
Total					**38**

Vani et al 2008; *J Fam Plann Reprod Health Care* 34:2-34

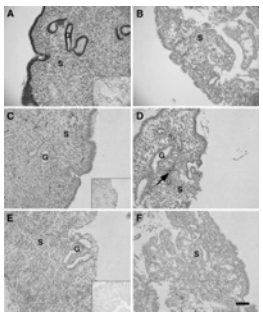
Sex steroid receptor expression in HRT exposed endometrium



Vani et al 2008; *J Fam Plann Reprod Health Care* 34:2-34

Sex steroid receptor expression in HRT exposed endometrium

HRT- no bleed | HRT- with bleeding



PR

GR

AR

Vani et al 2008; *J Fam Plann Reprod Health Care* 34:2-34

Mechanisms of bleeding on menopausal hormone therapy

(Vani et al 2008; *J Fam Plann Reprod Health Care* 34:2-34)

- In HRT users, during bleeding, trend observed towards decrease in PR and increase in GR expression in endometrial glandular cells.
- No differences in endometrial AR or ER expression.
- Endometrial steroid receptor expression in HRT users differs from that observed with normal menstruation and long-term progestogen-only administration.
- Different mechanisms likely involved in HRT-related unscheduled bleeding

Summary

- **Unscheduled endometrial bleeding is common among HRT users – leading to discontinuation of therapy.**
- **The mechanism of endometrial bleeding with hormone therapies (progestogen-only and hormone replacement) is likely regulated by sex steroids and their interactions with cognate receptors. The subsequent cascade of downstream events involving the endocrine, vascular and immune systems is complex.**
- **Since endometrial steroid receptor expression in HRT users differs from that observed with normal menstruation and long-term progestogen-only administration different mechanisms may be involved in HRT-related unscheduled bleeding**
- **A detailed knowledge of mechanisms of steroid regulation of endometrial function is essential for understanding how disturbances of endometrial structure and function may play a role in endometrial bleeding complaints.**

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