The menopause and its management, a revisit.
Mechanisms of irregular bleeding with hormone therapies

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ESHRE Campus workshop
Edinburgh, October 2008

“Mechanisms of irregular bleeding with hormone therapies”

Research Grant support from:
• 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
- 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 2003 J Clin Endocrinol Metab 88:5228-35)

The human menstrual cycle

Steroid receptor superfamily

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Steroid receptor superfamily

Clinical Orphan Receptors

Steroid hormones

Olefsky 2001; JBC 276:
Overview of steroid receptor expression in endometrium

<table>
<thead>
<tr>
<th>Protein expression</th>
<th>Proliferative</th>
<th>Secretory</th>
<th>Decidual</th>
<th>uNK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glands</td>
<td>Stroma</td>
<td>Glands</td>
<td>Stroma</td>
</tr>
<tr>
<td>PR</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ERα</td>
<td>+</td>
<td></td>
<td>+</td>
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<tr>
<td>ERβ</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ERβ1</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ERβ2</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>GR</td>
<td>-</td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

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
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Normal Cycle

Proliferative

Secretory

Proliferative

Secretory
Normal endometrial cycle

- **Unopposed oestrogen (E)** exposure promotes regeneration and proliferation post-menstrual phase.
- **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.
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. 85: 240 & 2563
Nayak et al 2000; J Clin Endocrinol Metab 85: 3442-52
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
Gene expression profiling of mid to late secretory phase endometrial biopsies from women with menstrual complaints

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

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
Heavy Menstrual Bleeding (HMB)

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

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**

<table>
<thead>
<tr>
<th>Normal proliferative</th>
<th>Down regulation of progesterone receptors with LNG IUS</th>
<th>PR A likely to be subtype mediating LNG action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month post LNG IUS insertion</td>
<td></td>
<td>Critchley et al. 1998</td>
</tr>
<tr>
<td>12 months post LNG IUS insertion</td>
<td></td>
<td></td>
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</tbody>
</table>

**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(flt-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)
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

Adapted from Vincent et al 2002 Hum Reprod 17: 1189

Co-localisation MMP-9 and leukocytes

neutrophils CD3+ T cells mast cells

MMP-9

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

Mast cells

MMP-1

MCT

MMP-1

MCT

MMP-1

Leukocytes in LNG-treated endometrium

Leukocytes in LNG-treated endometrium

Adapted from Vincent et al 2002 Hum Reprod 17: 1189

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

Mast cells

MMP-1

MCT

MMP-1

MCT

MMP-1

Leukocytes in LNG-treated endometrium
Progestin - only contraception (Norplant) and B-T-B
- enlarged, thin-walled vessels
- vascular fragility
- trend toward endometrial perfusion

TF and IL-8 Immunoreactivity

TF and IL-8 Immunoreactivity

TF IL-8

LNG-IUS LNG-IUS


Endometrial intracrinology

Ligand availability dependent upon local cellular enzymes.

- E₂ → 17βHSD 2 → E₁ 
- T → 17βHSD 2 
- E₁ → 17βHSD 5 
- Progesterone → 17βHSD 5 
- Pregnadiolone → 3βHSD 1 & 2 
- Aromatase → E₂

βHSD2 immunostaining

Burton et al. 2003
Hum Reprod 18:2610

17β HSD2 immunostaining enzyme expression inhibited with LNG

Mean and SEM

- Mean = 3
- P < 0.01

Duration of Intrauterine LNG

- 1 Month
- 6 Month
- 3 Month
- 12 Month
- Prot Sec 1 3 6 12 Months
- a
- a
- 0

- Prol Sec 1 3 6 12 Months
- 0
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 2001)
- Endometrial bleeding involves breakdown of endometrial vessels and overlying epithelium (Hickey, Menopause Int. 2007)
- Endometrial vascular breakdown is locally regulated (Smith, Hum Reprod 2006)
- 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.


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


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

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

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Women</th>
<th>Hormone replacement therapy</th>
<th>History of scheduled bleeding</th>
<th>Bleeding at time of endometrial biopsy</th>
<th>Biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26</td>
</tr>
</tbody>
</table>

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

Sex steroid receptor expression in HRT exposed endometrium

Sex steroid receptor expression in HRT exposed endometrium

HRT-no bleed HRT-with bleeding

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.

Acknowledgements

University of Edinburgh
- David Baird
- Anna Glasier
- Stephen Hillier
- Andrew Horne
- Ian Mason
- Pamela Warner
- Alistair Williams
- Past/present MD/PhDs
- Sharon Cameron
- Anja Guttinger
- Rebecca Jones
- Nicole Kane
- Oliver Milling-Smith
- Susheel Vani
- Julia Wilkens
- Oregon National Primate Research Centre, USA
- Robert M Brenner
- Ov D Slayden
- TAP Pharmaceuticals
- Kristof Chwalisz
- Cong Han
- MRC HRSU (Edinburgh)
- Henry N Jabbour
- Rodney W Kelly
- Philippa TK Saunders
- Lab/Team support
- Arima Lenthall
- Rosemary Calvert
- Jonathan Kay
- Bruce Leathem
- Claudia de la Senne
- Sandra Young
- Lab Equipment
- Grant support from:

- Current:
  - MRC Programme:G0500047; Project: G0600048
  - 1994 – 1996 MRC Project Grant G9405039F
  - 1997 – 2000 MRC Project Grant G9620138
  - 2000 – 2005 MRC Programme Grant G0000066
  - NIH CHD (RO1- HD - 43209-03)
  - CSO (CZB/4/513)
  - TAP Pharmaceuticals, USA

TAP Pharmaceuticals, USA