Nerve fibres in endometrium and lesions of endometriosis

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Endometriosis

- the presence of tissue, histologically similar to endometrium, outside the uterine cavity
- this tissue is functionally different from the eutopic endometrium
- the endometrium from women with endometriosis is functionally different from the endometrium of women without endometriosis

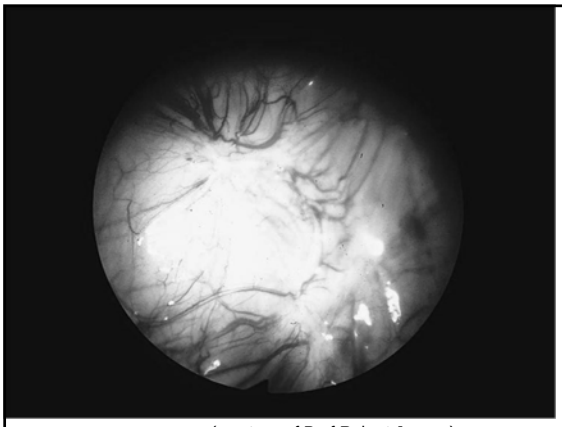
Symptoms of endometriosis:

(highly variable)

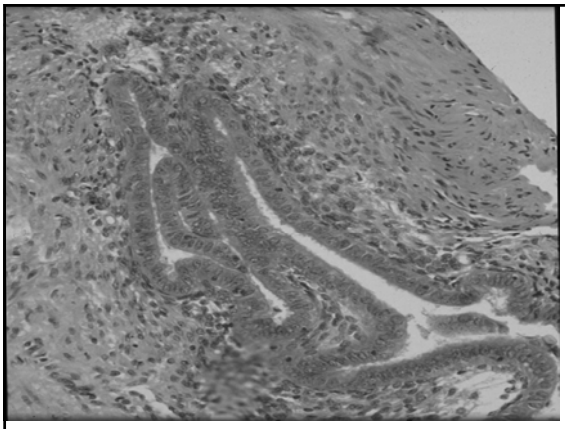
- none
- pain
  - secondary dysmenorrhoea
  - erratic and midcycle pain
  - dyspareunia and bowel symptoms, painful bloating
  - other pain symptoms (inc. neuropathic pain)
- menstrual
  - premenstrual spotting or heavy menstrual bleeding
  - vicarious menstruation
- infertility and ?miscarriage
- (malignant change)

Endometriosis and pelvic pain

- the relationship is unclear
- multiple causes of pelvic pain
- endometriosis is one cause
- some cases of anatomically mild and even severe endometriosis are not associated with obvious pelvic pain
- some cases of anatomically mild disease are associated with severe pain
- surgical and medical treatment only alleviate pain sometimes



(courtesy of Prof Robert Jansen)



Courtesy of Prof Robert Jansen

Is endometriosis an endometrial disease?

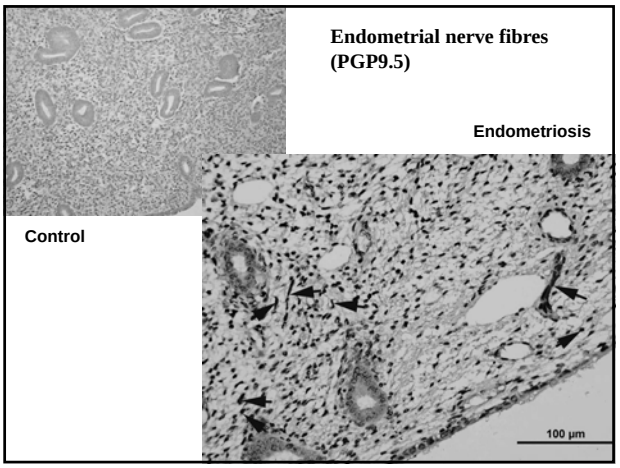
- increasing evidence suggests that endometriosis is a disease originating from abnormalities of endometrial function - and micro-structure
- apparent abnormalities of angiogenesis and lymph-angiogenesis
- multiple molecular abnormalities:
  - structural, metabolic and immune proteins (cytokeratins, integrins, heat shock proteins, actin, adhesion molecules, transcription factors, apoptosis, aromatase activity, oxidative pathways, etc)

Endometrial nerve fibres

- we began exploring the presence of sensory nerve fibres in the endometrium and myometrium of women with complaints of pelvic pain or menstrual symptoms
- we initially made the striking observation that ALL women with endometriosis have fine, sensory or autonomic, unmyelinated nerve fibres present in the functional layer of eutopic endometrium, while women without endometriosis NEVER have these nerve fibres

Fine nerve fibres in endometrium

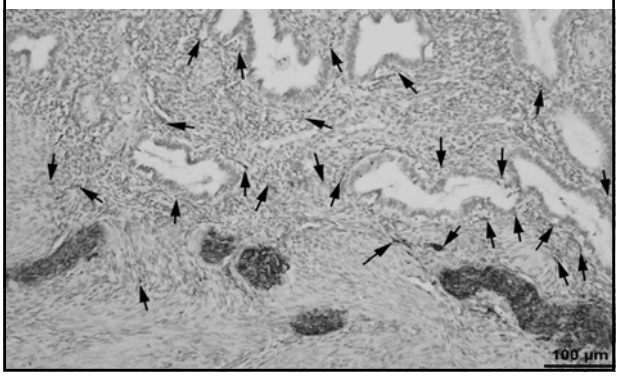
- immuno-histochemical localisation with specific tissue markers for nerve fibres (antibodies for molecules expressed by nerve fibres)
- pan-neuronal marker (PGP9.5) - specifically stains all nerve fibres
- stains for myelinated nerve fibres (neurofilament NF - stains A delta fibres)
- neurotransmitters and other markers for nerve fibres of different functions



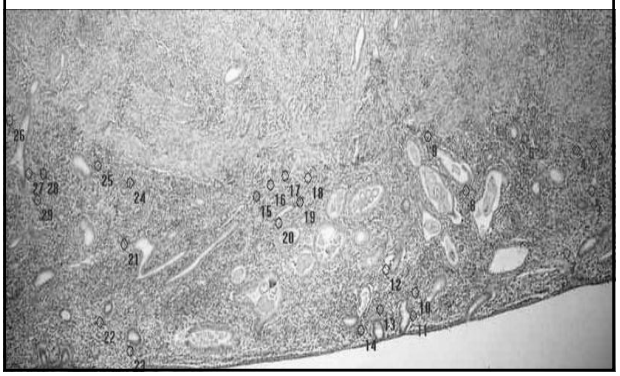
Sensory and autonomic C nerve fibres

- these fine unmyelinated nerve fibres in the functional layer of eutopic endometrium expressed:
  - vaso-intestinal peptide (VIP)
  - neuro-peptide Y (NPY)
  - substance P (SP)
  - calcitonin gene-related peptide (CGRP)

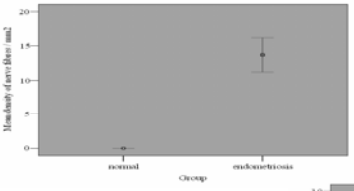
Basal layer of endometrium in biopsy-confirmed endometriosis (PGP9.5) (x200)



Nerve fibre distribution in full-thickness endometrium (biopsy-proven endometriosis; PGP9.5)

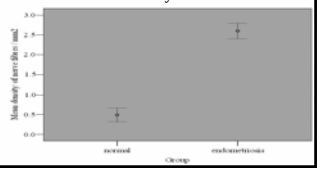


Mean (± SD) density of nerve fibres in the functional layer of endometrium and myometrium in women with and without endometriosis (PGP9.5)



Endometrium

Myometrium



(Tokushige et al, Hum Reprod 2006; 21: 782)

Identification of nerve fibre types

- identification of nerve fibre types is difficult
- these endometrial nerve fibres are probably a combination of sensory C and autonomic C fibres
- sympathetic fibres strongly express NPY, noradrenaline ("adrenergic") and ATP; but sometimes VIP and ACh [sympathetic fibres are controlled by cell bodies in the thoracic and lumbar regions]
- parasympathetic fibres strongly express VIP (and co-express NO synthase) and ACh ("cholinergic"), but sometimes NPY [parasympathetic fibres are controlled by cell bodies in the cranial and sacral regions]
- sensory fibres express Substance P and CGRP (± NF, VIP, NPY)

Visceral nerve fibre complexes

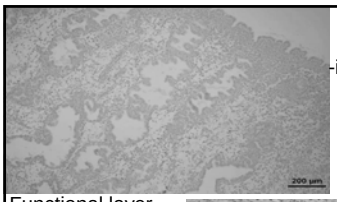
- afferents and efferents; branching fibres
- formation of plexuses
- considerable plasticity
- visceral sensory fibres include nociceptors - which may be polymodal
- nociceptors may be sensitised (changed threshold) in inflammatory conditions
- mostly unmyelinated C fibres (transmission at 1 - 2 metre per sec)
- few A delta fibres transmitting at 10 m/sec

Nociceptors

- 'silent' receptors which do not respond to 'normal' stimuli
- sensory nerve fibre receptors which are responsive to 'noxious' stimuli - stimuli which have the potential to do harm; trigger a reflex
- send signals which initiate the sensation of pain
- in visceral organs they tend to respond to:
  - excessive pressure
  - excessive stretch
  - 'inflammatory' processes
  - a range of 'injurious' chemical substances

Fascination with what may be happening to these fibres during menstruation

- some fibres lie very close to the epithelial surface
- are these fibres damaged and partially 'shed', then remodel?
- do they remain intact?
- is there a significant re-growth each cycle?
- are there other examples of rapid remodelling of nerve fibres?
- what do we know of uterine nerve plexus plasticity?
- are these nociceptors sensitised by menstrual breakdown?

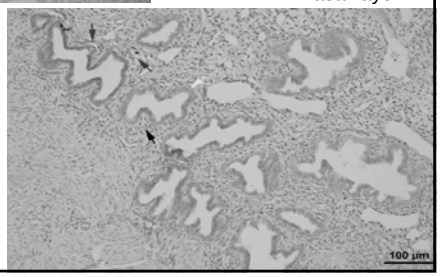


Nerve growth factor (NGF)
-immuno-histochemistry (DAB)

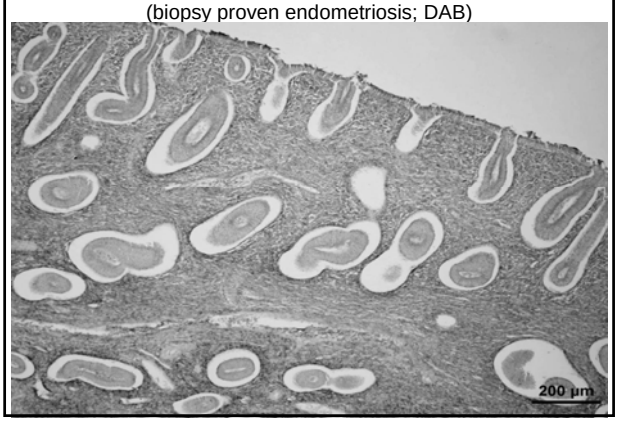
No endometriosis

Basal layer

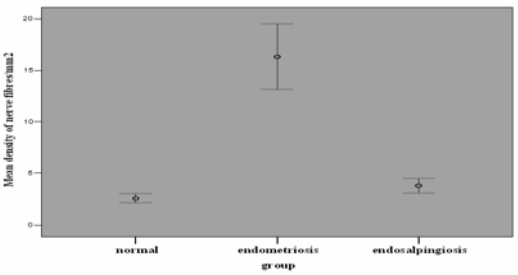
Functional layer



Nerve growth factor in eutopic endometrium
(biopsy proven endometriosis; DAB)



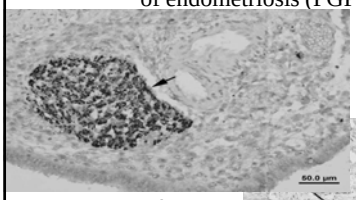
Group comparison of mean (±SD) density of nerve fibres (PGP9.5) in peritoneal endometriosis, endosalpingiosis and normal peritoneum.



Deep pelvic nodules: mean nerve density 66.0 ± 43.5/mm² (range 2 to 300!)

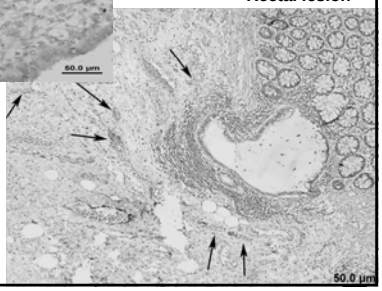
(Tokushige et al, 2006; Wang et al 2009)

Nerve fibres in peritoneal and deep infiltrating lesions of endometriosis (PGP9.5; fast red)



Large nerve trunk
(multiple different types of nerve fibres)

Rectal lesion



What are these nerve fibres actually doing?

- nociceptors for detection of painful stimuli
- what are the pain stimuli?
  - Role of NGF? Prostaglandins? (up-regulated)
  - Bradykinin? Histamine? (activated mast cells?)
  - "inflammatory sensitisation"; oestrogen sensitisation
- autonomic fibres
  - vascular control
  - epithelial secretory functions
- unknown functions

Implications of these findings

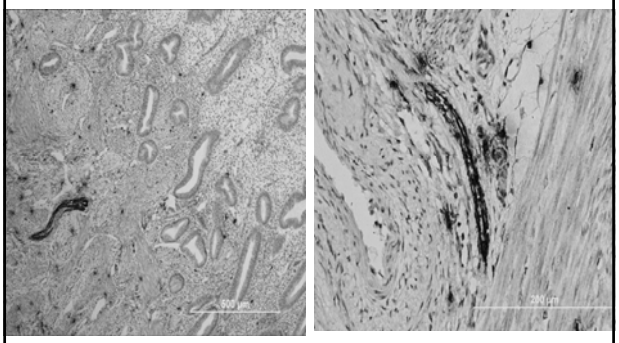
- many new directions to understand the roles and functions of these nerve fibres
- how do different nerve fibres relate to symptoms?
- what is the role of the nerve fibres in pathogenesis of endometriosis?
- what happens to them during treatment?
- potential for the development and delivery of long-acting nociceptor blockers
- potential for developing a less invasive means of diagnosing endometriosis (than laparoscopy)
- diagnosis of endometriosis in adolescents before typical manifestations of the disease

Relationship between nerve fibres and immune cells in peritoneal lesions

- **macrophages**:
  - low density of macrophages (< 51/mm^2) is associated with low density of nerve fibres (16.0 ± 17.1/mm^2)
  - high density of macrophages (> 50/mm^2) is associated with high density of nerve fibres (49.3 ± 34.9/mm^2); $p < 0.025$
- **mast cells and dendritic cells** may also have an anatomical relationship with nerve fibres in basal endometrium

(Berbic, Schulke, Al-Jefout et al, 2009; Tran, Berbic et al 2009: in press)

Relationship between activated mast cells and nerve fibres in eutopic endometrium and myometrium

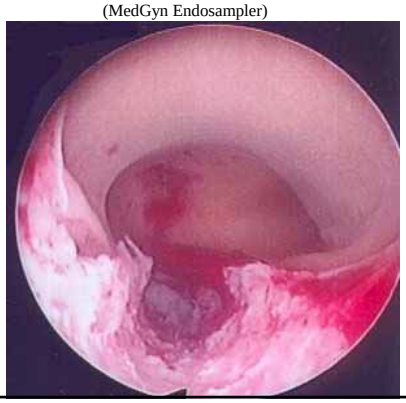


(Al-Jefout et al; Fertil Steril, in press)

Diagnosis by endometrial biopsy

- we set up a pilot trial to assess the presence of endometrial nerve fibres in endometrium of women with and without laparoscopically confirmed endometriosis
  - endometrial suction biopsy (careful technique)
  - full curettage
  - immuno-histochemistry with PGP 9.5
- 20 women with endometriosis
- 18 women without endometriosis

Hysteroscopic view after endometrial biopsy
- secretory phase
(MedGyn Endosampler)



Diagnosis by endometrial biopsy - pilot study findings

- findings identical for endometrial biopsy and full curettage
- ALL women with endometriosis (20) had recognisable nerve fibres
- NO women without endometriosis (18) had any nerve fibres detected
- 100% sensitivity and 100% specificity

(Al-Jefout et al, Am J Obstet Gynecol 2007; 197: 578)

Diagnosis of endometriosis by endometrial biopsy: a prospective double-blind trial

- Total patients: n = 99 women; (64 with endometriosis and 35 without endometriosis)
- Single endometrial marker; at any stage of the cycle
- Symptoms:
  - pain symptoms alone (n = 52),
  - infertility alone - no pain (n = 6),
  - pelvic pain and infertility (n = 41)
    - mild pain 2
    - moderate pain 39

(Al-Jefout et al, submitted)

Overall detection of endometrial nerve fibres in double-blind trial

- Small sensory C-nerve fibers were detected in 63 out of 64 women in whom endometriosis was surgically diagnosed.
- Endometrial nerve fibres were detected in 6 cases in whom endometriosis was not confirmed on laparoscopic inspection (n = 35)

(Al-Jefout et al, submitted)

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(Al-Jefout et al, submitted)		Endometriosis diagnosis at laparoscopy		Total
		Yes	No	
Endometrial nerve fibres present	Yes	63	6	69
	No	1	29	30
Total		64	35	99
Specificity		83 %		
Sensitivity		98 %		
Positive predictive value		91 %		
Negative predictive value		96 %		

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Discordant results

- We found only one case (age 43) with no detectable nerve fibres, but clear evidence of stage IV endometriosis at laparoscopy
- Cases (n = 6) with positive biopsy for nerve fibres but negative endometriosis at laparoscopy:
  - Four of these cases had pain and infertility.
  - One case had a single spot of adhesions on the Pouch of Douglas which was not considered convincing for endometriosis.
  - One case had had endometriosis diagnosed and removed at laparoscopy seven years previously, but no evidence of active endometriosis was found at recent laparoscopy

(Al-Jefout et al, submitted)

Effects of hormonal therapy on endometrial and endometriotic nerve fibres

(in women with some persisting symptoms)

- in eutopic endometrium
  - in only 3 out of 26 women were nerve fibres still detectable in the functional layer
  - residual nerve fibres only stained with VIP and NPY
  - very weak staining for NGF and NGFRp75
- in ectopic endometriotic tissue
  - in all of 18 peritoneal biopsies examined so far (from women on progestogens or COCP), nerve fibres were still present but at reduced density

(Tokushige et al, Fertil Steril 2008; 90: 1589)

What are the implications for future treatment?

- hormonal therapies usually suppress most endometrial nerve fibres
- hormonal therapies reduce but do not eliminate nerve fibres from endometriotic lesions
- LNG-IUS very effectively suppresses endometrial nerve fibres and minimizes endometriosis recurrence
- LNG-IUS and subdermal etonogestrel are more effective than either alone (local and distant)
- progesterone receptor modulators may be effective
- eliminating aromatase may be of additional value
- anti-nerve growth factor therapies may be effective

Conclusions

- women with endometriosis and pelvic pain almost always have fine nerve fibres present in the functional layer of endometrium (and greatly increased in myometrium)
- women without endometriosis almost never have these nerve fibres
- these nerve fibres may play a role in pain generation
- the presence of these nerve fibres may allow reliable diagnosis without recourse to laparoscopy
- the presence of these nerve fibres may predate the development of endometriotic lesions and symptoms
- there may be important implications for understanding the impact of treatments and for evolving new treatments

Collaborators

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- Dr Natsuko Tokushige
- Dr Frank Manconi
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- Dr Wang Guoyun
- Mr Paul Tran
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- Ms Marina Berbic
- Prof Peter Russell
- Dr Michael Cooper
- Prof Janet Keast