



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Animal Models of Endometriosis: replicating the aetiology and symptoms of the human disorder.

Citation for published version:

Simitsidellis, I, Gibson, D & Saunders, P 2018, 'Animal Models of Endometriosis: replicating the aetiology and symptoms of the human disorder.', *Best Practice and Research: Clinical Endocrinology and Metabolism*. <https://doi.org/10.1016/j.beem.2018.03.004>

Digital Object Identifier (DOI):

[10.1016/j.beem.2018.03.004](https://doi.org/10.1016/j.beem.2018.03.004)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Best Practice and Research: Clinical Endocrinology and Metabolism

Publisher Rights Statement:

Author's peer reviewed manuscript as accepted for publication.

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Accepted Manuscript

Animal Models of Endometriosis: replicating the aetiology and symptoms of the human disorder

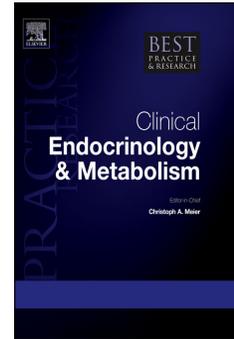
Ioannis Simitsidellis, PhD, Douglas A. Gibson, PhD, Philippa TK. Saunders, PhD

PII: S1521-690X(18)30046-0

DOI: [10.1016/j.beem.2018.03.004](https://doi.org/10.1016/j.beem.2018.03.004)

Reference: YBEEM 1200

To appear in: *Best Practice & Research Clinical Endocrinology & Metabolism*



Please cite this article as: Simitsidellis I, Gibson DA, Saunders PT, Animal Models of Endometriosis: replicating the aetiology and symptoms of the human disorder, *Best Practice & Research Clinical Endocrinology & Metabolism* (2018), doi: 10.1016/j.beem.2018.03.004.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Animal Models of Endometriosis: replicating the aetiology and symptoms of the human disorder.

Ioannis Simitsidellis PhD, Douglas A Gibson PhD, Philippa TK Saunders PhD*

5

Medical Research Council Centre for Inflammation Research, The University of Edinburgh,
Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ. UK

*corresponding author

10 Email addresses:

Ioannis Simitsidellis: ioannis.simitsidellis@ed.ac.uk

Douglas Gibson: d.a.gibson@ed.ac.uk

Philippa Saunders: p.saunders@ed.ac.uk

15

Abstract

Endometriosis is a chronic incurable disorder that affects 1 in 10 women of reproductive age:
20 associated symptoms include chronic pain and infertility. The aetiology of endometriosis
remains poorly understood but patients, clinicians and researchers are all in agreement that
new non-surgical therapies are urgently needed to reduce the severity of symptoms.
Preclinical testing of drugs requires the development and validation of models that
25 recapitulate the key features of the disorder. In this review we describe the best-validated
animal models (primate, rodent, xenograft) and their contributions to our understanding of the
factors underpinning the development of symptoms. We consider the evidence that these
models have provided the platform for identification of new therapeutic interventions and
reflect on future directions for research and drug validation.

30 **KEY WORDS:** endometriosis, pain, infertility, neuroangiogenesis, oestradiol, inflammation,
macrophage.

General Practice Points

- 35
- Endometriosis is a chronic, incurable hormone-dependent disorder characterized by the presence of ‘lesions’ which resemble fragments of endometrial tissue at extra-uterine sites
 - Symptoms include chronic pain and infertility: not all women with lesions have symptoms/all symptoms.
- 40
- Endometriosis only develops spontaneously in women and menstruating primates.
 - Endometriosis is considered to be an oestrogen-dependent inflammatory disorder.
 - Endometriosis can be induced experimentally in primates and rodents leading to formation of lesions that resemble those found in women.
 - Induction of ‘lesions’ allows investigation of specific processes/targets, but as yet, no
- 45
- model completely recapitulates all aspects of the human disorder.
 - In animal models inhibition of oestrogens or inflammatory signals can reduce lesion growth and pain symptoms.

50

1. Introduction.

Endometriosis is an incurable disorder that is estimated to have an impact on the health and wellbeing of ~1 in 10 women of reproductive age (1). The defining characteristic of the disorder is the presence of tissue fragments (lesions) that contain stromal, epithelial and inflammatory cells mimicking the appearance of uterine (eutopic) endometrium. The time to diagnosis is estimated to be 7 years from the time the symptoms first appear (2) and definitive diagnosis is only achieved by a surgical laparoscopy. Depending upon the number, location and appearance of the lesions, three broad subtypes have been postulated; peritoneal, ovarian and deep infiltrating. These phenotypes are further classified, according to the criteria set by the American Society for Reproductive Medicine into stage 1 (minimal), stage 2 (mild), stage 3 (moderate) or stage 4 (severe) (3).

One of the most debilitating symptoms of endometriosis is chronic pain that may be constant or associated with fluctuations in the menstrual cycle. In their 2007 study, Vercellini et al (4) analysed the association between patient clinical characteristics, lesion type, disease stage and severity of pain symptoms in a cohort of 1054 women having surgery for endometriosis and concluded that ‘the association between endometriosis stage and severity of pelvic symptoms was marginal and inconsistent’, a finding that has been replicated in other reports on smaller groups of women with endometriosis (5). Landmark studies by Berkley and Stratton have redefined the relationship between lesions and pain symptoms by considering the evidence that endometriotic lesions can develop their own nerve supply, thereby creating a direct and two-way interaction between lesions and the CNS [reviewed in (6)]. Asante and Taylor coined the term ‘neuroangiogenesis’ to describe the extensive cross-talk between nerves and blood vessels by which lesions develop a unique local neuronal and vascular supply (7). It is

also notable that recent studies have reported that in addition to physical pain symptoms, psychological factors such as stress, anxiety and depressive symptoms all have a negative impact on the health-related quality of life of women living with endometriosis (8). These findings have stimulated a great focus on the mechanisms that might alter pain perception in women with endometriosis and prompted an increase in the efforts to develop relevant preclinical models to investigate mechanisms and test potential therapies (6) (see section 5).

The incidence of endometriosis may be as high as 50% in women presenting at infertility clinics and a recent paper reported that the chance of having a baby even with IVF or ICSI was significantly reduced in women with endometriosis compared to women without the condition (9).

Why women develop symptomatic endometriosis remains something that is the subject of intense research interest. Whilst the most widely accepted theory for the formation of lesions is that of ‘retrograde menstruation’ that was proposed by Sampson 90 years ago (10), numerous commentators have concluded that this cannot explain why some but not all women develop symptomatic lesions and factors including the peritoneal environment, reduced immune surveillance, persistence of stem cells and oestrogenic stimulation, all being considered (1,11-13). Currently there is an increased interest in using genome wide association studies to better understand the risks/aetiology of endometriosis. A concerted international effort is underway using many thousands of samples (14). The hope is that these studies may help identify targets to improve diagnosis.

1.1. Priorities for Research in Endometriosis

The World Endometriosis Society [WES, <http://endometriosis.org/>] has brought together many of the key stakeholders interested in advancing understanding of endometriosis and its

treatments. It works closely with WERF (the World Endometriosis Research Foundation, <http://endometriosis.ca/research/werf/>), which was formed in response to the need to accelerate research activities related to improving understanding of the aetiology of the disease and foster development of new therapies. These groups have brought together researchers and clinicians and published a series of papers detailing protocols for collection of tissues and fluids for research as well as research priorities (15). Of note, many of the research priorities include recommendations building on the use of animal models. A recent exercise in priority setting that sought the views of patients, their supporters, researchers, as well as those involved in their care (clinicians and allied health professionals) has published a ‘Top 10’ of research priorities (16). Tools to address these challenges include extensive use of human tissue resources (recently reviewed in (17)), as well as a range of animal models which are reviewed below.

2. Methodology.

A search was conducted on Medline (Pubmed) using the search terms ‘animal model’ and ‘endometriosis’ on 15/8/17. All abstracts were read to verify the mention of an animal model and 747 papers were identified for further interrogation. Notably, there were a number of papers published in 2016/7 consistent with the recent identification of research priorities related to the aetiology of the disorder as well as the evaluation of drugs and other agents in preclinical studies. A further search on 15/10/17 did not result in additional novel models. A number of reviews previously published on this topic were also read to any historical primary publications that were not found during the Pubmed search (17-19).

3. Animal models of endometriosis

125 In a milestone review, Ruth Grummer highlighted the range of species used during the 1980's
and 1990's in animal models for endometriosis research, identifying a number of small
laboratory animals including rats, mice, rabbits and hamsters (18): readers are referred to that
paper for a historical perspective. For the purposes of this review we have focused on models
that have been shown to recapitulate key features of the human disorder highlighting recent
130 adaptations which appear to offer promise for understanding the aetiology of the disorder or
as a platform for preclinical testing.

3.1 Primates

Menstruating primates can develop spontaneous disease although detection rates are generally
reported as being low, in part because females are often only detected when the severity of
135 their symptoms results in obvious changes in behaviour. Spontaneous disease with lesions
resembling those in women was reported in cynomolgus monkeys in a large breeding facility
in Japan (20,21). In these animals, symptoms included decreased food consumption (21); in a
group of 27 retired breeders the majority had cystic lesions and co-incident adenomyosis was
also detected (20). Dehoux et al studied 41 baboons at a Kenyan primate facility using
140 laparoscopy to prospectively monitor them for 11 months (22). In that time only two animals
developed spontaneous disease (4.8%). They subsequently used endocervical canal to
encourage flow of shed endometrium into the peritoneal cavity but reported that only 5 out of
30 females developed lesions after 9 months. In the conclusion to their paper they speculated
that baboons may have retained some mechanisms that promote peritoneal healing now lost in
145 women and this may limit their use in studies on endometriosis.

However, other groups, notably those led by d'Hooghe (23,24) and Fazleabas (25,26) have
established reproducible protocols to induce lesions in the peritoneum of baboons based on
autologous inoculation of menstrual tissue (summarized in Figure 1) (27). In another study,
deep nodules have been induced in the baboon model with results suggesting that cell

150 migration played an important role in the invasion process of deep lesions in this species (28).
Endometriosis has also been artificially induced in *Cynomolus* monkeys (29) and the non-
menstruating Common Marmoset monkey (30): the latter is a small primate and may be an
attractive option for testing compounds *in vivo*. It has been reported that attempts to induce
155 lesions in rhesus macaques were unsuccessful, however, only three rhesus macaques were
used in that study due to ethical considerations and the rhesus macaques were not cycling so
proliferative stage endometrium was used for lesion induction rather than menstrual tissue
(31).

3.2 Rats

In 1985 Vernon and Wilson explored different ways of inducing 'endometriosis' lesions in
160 intact rats and reported that autotransplanting small fragments of uterine horns (myometrium
and endometrium) onto the peritoneum resulted in formation of cystic lesions and impaired
fertility (32). This model has been adopted and adapted by many groups, most notably by
Berkley, who refined the method to suture the small pieces of uterus (or fat) onto mesenteric
arteries, abdomen, and ovary (33-35). This protocol has been widely adopted and proved
165 valuable in demonstrating innervation of lesions (34) and informed our understanding of the
pathways that may be activated during pain responses (6,36) (see more details in section on
models of pain).

Variants on this model include the stitching of autologous tissue onto the peritoneal wall (37)
and a new model that recapitulates the phenotype of colo-rectal endometriosis (38). In a more
170 radical rat model autologous uterine tissue has been grafted onto the gastrocnemius muscle
(39); although this model does not allow for an evaluation of the role of the peritoneum it
shows promising for testing mediators of sensory nerve activation.

3.3 Mice

175 Models have been developed in mice to take advantage of access to transgenic lines in which genes have been selectively ablated, including those implicated in oestrogen signaling (40-42), inflammatory processes (43) and cells/endometrial tissue expressing fluorescent proteins under the control of cell-specific (44) or ubiquitous promoters (45,46). In allogeneic mouse models uterine tissue is recovered minced/dissected and either stitched back onto the intestinal mesenteric vessels (47), peritoneal wall or injected into the peritoneal cavity (48).

180 Pelch et al published a video showing the methodology adapted from the rat, in which tissue is surgically transplanted onto the intestinal mesentery, which is particularly useful as a primer for researchers unfamiliar with the method (49). Authors have also reported that recovery of tissue at estrus (highest oestrogen levels) is advantageous: in some cases it has been reported that lesions develop cystic structures similar to those reported in the rat (47).

185 More commonly, a syngeneic approach is adopted with the uterine tissue (donor) being introduced into a different mouse (recipient): a number of variations of this method have been reported (42,44,45,50). Standard methods include removal of uterine horns at diestrus, longitudinal opening of the horn to expose the endometrial lining which was cut into small

190 fragments e.g. using a biopsy punch and suturing onto the peritoneal wall (41,42). Hirata et al used GFP-expressing mice as donors and injected minced endometrial fragments into the peritoneal cavity of ovariectomised mice supplemented with E2 (51), thus allowing for easier identification of lesions. In an elegant series of experiments Ferrero and colleagues used decidualised donor endometrial tissue from mice which they recovered and labeled with

195 mCherry fluorescent protein *ex vivo* prior to introducing them into different sites in recipients (50). Lesions were formed that had epithelial cells forming glands, evidence of inflammatory cells, collagen deposits and new blood vessel formation (50). Their results showed that

'lesions' can be induced in many different locations and provide a framework for non-invasive monitoring of lesion growth based on imaging.

200

In an attempt to more closely model the mechanisms of 'retrograde' menstruation and recapitulate the successful induction of lesions in menstruating primates, like the baboon, Greaves et al adapted the syngeneic model to use donor tissue from mice in which endometrial tissue shedding had been induced by removal of a progesterone pellet following a
205 decidual stimulus (52). The menstrual tissue was injected into the peritoneal cavity of ovariectomised mice who were given an E2 implant resulting in the formation of lesions which phenocopied those in women (44) (an overview of the methodology is provided in Figure 2). As with other models reporting results from intraperitoneal injection of endometrial tissue, the number and location of lesions in each recipient mouse may vary, typically
210 between one and three lesions located in the peritoneum or fat. To overcome this limitation the model was adapted by researchers in Leuven using laparoscopy to position lesions on the peritoneum (53). This approach seems to offer advantages over the other peritoneal models that may induce surgery-associated trauma, adhesion formation or variations in the location/number of lesions, however, it does require access to specialized equipment which
215 may not be available to all researchers.

An alternative model for induction of lesions using steroid-manipulated, menstrual-like endometrium employs K-ras(G12V/+)/Ah-Cre(+/+)/ROSA26R-LacZ(+/+) mice as donors and gonad intact wild type mice as recipients (54). Tissue was injected into a subcutaneous
220 pocket and allowed to develop without exogenous E2 supplementation.

Immunohistochemistry confirmed that as in human endometriosis, there was invasion and

activation of fibroblasts, endothelial cells, and macrophages and collagen deposition in the lesions (54).

3.4 Human cell and tissue xenograft models

225 A number of groups have employed a combination of mice (immunosuppressed recipients, nude, NOD/SCID, Rag2/gammaC) with human cells or tissue samples to model lesions. These have the advantage of using a relevant tissue type (human) but use of immunocompromised mice limits any modeling of the non-endometrial inflammatory contribution to lesion formation/development. Jafarabadi and colleagues demonstrated that

230 subcutaneous transplantation of isolated human endometrial tissue or cultured primary human endometrial mesenchymal (CD90+) cells both result in the formation of endometriotic lesions in gamma irradiated mice (55). Xenografts of mixed populations of human immortalized endometriosis epithelial and stromal cells can also develop into lesions within the peritoneum which were able to reorganise, proliferate, form glands and express regulatory molecules

235 including ERalpha, prostaglandin receptors and COX-2 (56). Hull and colleagues have used a refinement of this model in which human endometrial tissue fragments are xenografted into nude mice (57-59). In other studies, whilst human tissue grafts initially retained their own blood vessels, over time, they connected to the murine vasculature and became functional (60). New xenograft models including use of recipients that have been 'humanized' so that

240 they contain human immune cells are under development but as yet there are no peer-reviewed publications reporting results. Such refinements may enhance the utility of xenografts for preclinical testing of drugs.

4. Contributions of Animal Models to Processes that may contribute to symptoms of pain and infertility.

4.1 Immune cells and inflammation

The peritoneal fluid of women with endometriosis contains higher concentrations of pro-inflammatory cytokines and prostaglandins and changes in immune cell complement (reviewed in (12)). There is also evidence that proinflammatory molecules, including
250 prostaglandins (e.g. PGF2 α , PGE2) that are produced by macrophages and other endometrial cells, act via their cognate receptors (FP, EP1-4) within endometrial lesions. In a xenograft model (nude mice, human tissue fragments), treatment with the FP antagonist AL8810 led to a decline in the number and size of lesions and downregulation of MMPs and VEGF (61). In other studies authors have used athymic immunocompromised (RAG2/gamma c) mice that
255 lack T cells and implanted these with human cell lines derived from an active peritoneal lesion. They showed that treatment of the mice with specific EP2 and EP4 antagonists decreased angiogenesis and innervation of the resulting xenografts (62). This model has limitations as reports using a baboon model suggest the presence of endometriosis in intact animals may have specific impacts on Treg cells (63). Notably this is a T cell subset which
260 has been implicated in exacerbating endometriosis in women (64) and which merits further study.

Allogeneic and syngeneic rodent models overcome the limitations of the xenograft models in immune deficient hosts and have been used extensively to investigate the role of immune
265 cells and inflammatory mediators in the development of lesions. For example, using uterine allografts in E2-supplemented mice, Lin et al (65) reported infiltration of neutrophils into ectopic tissue on days 1-4 and whilst macrophage numbers did not change, secretion of macrophage-derived angiogenic factors increased. A role for alternatively activated
macrophages in the establishment of lesions has been explored using a mouse model in which
270 macrophages were depleted in recipients. Depletion resulted in lesions that failed to organize and develop whereas adoptive transfer of alternatively activated macrophages dramatically

enhanced their growth (66). When Greaves et al (44) used Macgreen mice (mice with GFP expressed in CSF1R-positive cells; predominantly monocytes and macrophages), they reported that macrophages from both the peritoneal cavity (recipients) and donor
275 endometrium can contribute to the resident immune cell population of the lesions. In a syngeneic mouse model, Azuma et al reported that bi-weekly intraperitoneal injection of the pro-inflammatory mediator lipopolysaccharide (LPS) was associated with increased cell proliferation, increased immune cells and favoured the development of lesions (67). Guo and colleagues have also provided novel evidence that platelets are an important source of TGF-
280 β 1, which can in turn have immunosuppressive effects (68). They used a previously validated mouse model in which endometrial fragments from syngeneic donors are injected into the peritoneal cavity (69) of E2 treated recipients and showed platelet depletion in recipients significantly reduced lesion size, cell proliferation, VEGF and TGF- β 1 in the resultant 'lesions' (68).

285 4.2. Oestrogens and oestrogen receptors

Studies in animal models have complemented and extended evidence from human studies that endometriotic lesions contain higher concentrations of oestrogens, increased amounts of oestrogen biosynthetic enzymes and multiple oestrogen receptor-positive cells (70). These results were extended by analysis of lesions in the Baboon, where it was reported that ER β
290 was rapidly up-regulated in newly formed lesions but upregulation of P450 aromatase was not detectable until 10 months after induction (71), showing the value of a model where lesions can be studied from time of induction as a complement to human studies where lesions are likely to be long established at the time of removal.

295 In their mouse model, Greaves et al reported that lesions formed 3 weeks after induction recapitulated features of the human disease with overexpression of ER β (44). In further

studies they reported the macrophages in lesions were ER β -positive (72) and that treatment of mice with induced endometriosis with the ER β -selective agonist DPN can alter growth of lesion-associated blood vessels and nerves (73). Burns et al reported that compared with WT lesions transplanted into WT hosts, WT lesions were proliferative in ER α KO recipient mice but showed decreased inflammatory responses upon E2 treatment (40). The Katzenellenbogen group has used mice with peritoneal lesions induced using endometrial fragments stitched onto the peritoneal wall to explore the role of oestrogens/oestrogen receptors in lesion formation and associated inflammatory responses (41,42). They also used genetically modified mice (both donor and recipient) to explore a potential role for the ER coregulator, repressor of ER activity (REA) in the pathogenesis of the disorder. Their studies in mice, which were complemented by *in vitro* studies on human stromal cells, suggest this factor modulates the oestrogen-dependent interplay between cell types within the lesions (41).

4.3. Neuroangiogenesis

The Taylor group recently reviewed evidence that ectopic endometriosis lesions are able to recruit their own blood supply and advanced the hypothesis that 'neuroangiogenesis' (the coordinated growth of new blood vessels and nerves) is important for survival and growth of lesions and the associated pain experienced by many patients (7). Evidence for neuroangiogenesis in lesions of rats and mice is consistent with the data in women. Notably, in rats, mesenteric lesions develop both autonomic and sensory innervation (34) which becomes functionally active 2-3 weeks post transplantation (74). In the same species, endometrial tissues transplanted onto the gastrocnemius muscle also show evidence of neuroangiogenesis (39). Greaves and colleagues used their mouse model of endometriosis to explore potential regulators of neuroangiogenesis demonstrating a role for oestrogen receptor activation in regulation of the axonal guidance molecule Slit3: this factor appeared to promote

angiogenesis but to decrease neurogenesis providing a link between steroids and neuroangiogenesis (73) which merits further investigation.

Practice Points

- 325
- In animal models where lesions or cysts consisting of endometrial tissue fragments/cells are induced within the peritoneum growth of both new blood vessels (angiogenesis) and nerves has been detected.
 - The presence of lesions is associated with changes in the concentrations of inflammatory mediators in peritoneal fluid.
- 330
- Lesions contain a diverse population of immune cells including macrophages that may be derived from either the implanting tissue or the environment in which it becomes implanted.
 - Lesions have increased expression of oestrogen receptors which are implicated in regulation of proliferation and inflammation.

335

5. Studies using Animal Models to Investigate Mechanisms and Treatments for Pain

Pain associated with endometriosis has a significant impact on the quality of life of patients and development of new and effective therapies is a research priority that requires the

development of appropriate pre-clinical models of endometriosis. Measurement of 'pain' in

- 340
- animal models depends upon the evaluation of behavioural responses which may be stimulus-dependent (e.g. von Frey, hotplate, vaginal distension) or spontaneous (e.g. grooming, burrowing) with the latter being accepted as a key challenge for researchers (75).

The rat model of endometriosis, in which cystic lesions are formed on the intestinal mesentery (33,34,76), has been used extensively to explore the relationship between the presence of

- 345
- lesions, nerve cell growth and pain pathways (reviewed in (6)). The Berkley group has shown

that rats develop vaginal and abdominal muscle hyperalgesia 4-5 weeks after transplant (74) which is exacerbated by oestrogen (76), can be prevented if lesions are removed before they become innervated (74) and involves COX enzyme activation (77). This model has provided a platform for testing drugs that might alleviate pain in women with some promising results achieved with antagonists directed against cannabinoid (CB1) receptors (78) and anti-inflammatory drugs (77,79). In a recent study, Hernandez et al (80) used the model to test pain perception (hyperalgesia – hot plate; allodynia – Von frey) and showed that swimming exercise improved some symptoms.

Levine and colleagues used an alternative rat model in which cystic endometriosis-like lesions developed a local blood and nerve supply following surgical implantation onto the leg (gastrocnemius) muscle: in this model they performed measurements of mechanical hyperalgesia (39). They also explored the impact of surgical excision (81) and leptin (82) using a digital force transducer and single-fibre electrophysiology following stimulation with Von Frey fibres. This model allows for a more direct readout of nerve fibre activity in a lesion than other models but does not model the impact of the peritoneal environment on nerve activity.

Mouse models have not been used as extensively as rats but recent advances include a study by Greaves et al who showed the presence of peritoneal lesions resulted in upregulation of prostaglandins, changes in gene expression in dorsal root ganglia and the CNS, and altered spontaneous behavior and increased sensitivity to Von Frey testing consistent with abdominal hypersensitivity (83). Notably, when drugs targeting PGE2 receptors (EP2, EP4) were administered, an orally active selective EP2 antagonist was the most effective in abrogating pain responses. These studies complement those in the rat mesenteric cyst model where a

positive correlation has been demonstrated between peritoneal fluid PGF2a/PGE2 and vaginal hyperalgesia (84).

Practice Points

- Surrogate measurements of 'pain' responses include assessment of both spontaneous and evoked responses.
- In rats the presence of endometriosis-like cysts on the mesentery within the abdominal cavity is associated with development of both vaginal and abdominal hyperalgesia.
- Rats have been used to directly measure nerve cell activity by establishing endometrial cysts on the leg muscle.
- Appropriate blinding and randomization in behavioral monitoring is required to minimize effects of operator bias.

6. Studies using Animal Models to Investigate Mechanisms and Treatments for Infertility

A number of factors have been cited to explain the raised incidence of sub/in-fertility in women with endometriosis which were summarized by the Practice Committee of the American Society for Reproductive Medicine (ASRM) in 2012 (85). These included changes in ovarian and endometrial (eutopic) tissue function, as well as changes in the concentrations of pro-inflammatory mediators in peritoneal fluid (12,13). Fertility can only be assessed in females where both ovaries and lesions are present and as many models favour supplementation with E2 to promote lesion growth these are limited in number.

Cynomolgus monkeys with auto-transplantation of endometrium or adipose tissue only developed infertility when endometrium was used (29). In this case infertility was associated with luteinized un-ruptured follicles, luteal phase defects, and pelvic adhesions. The baboon

model of endometriosis has been used very effectively to conduct longitudinal sampling of endometrium and lesions with studies highlighting changes in the eutopic endometrium that only occur many months after induction of lesions. In these studies, endometrium was collected during the midsecretory phase between 1 and 16 months after introduction of autologous endometrium into the peritoneum and compared with endometrial tissue from controls, a clear time-dependent change in the patterns of gene expression was recorded (see Figure 3) (86). One notable change was in the expression of HOXA10 (87), a progesterone-regulated gene implicated in fertility that appeared to be differentially methylated in endometriosis females.

405

In a recent paper, Bilotas et al (88) induced lesions by syngeneic transplantation onto the bowel mesentery of mice and recorded mating and fertility parameters 4 weeks later. They reported a decrease in fertility rate and increased IL-2 in the peritoneal fluid with reduced lesion size in pregnant females, consistent with pregnancy-associated hormonal changes.

410

Studies in rats with induced endometriosis have also revealed that rats with lesions had an adverse ovarian phenotype including reduced numbers of follicles and luteinized un-ruptured follicles (89). In follow-up studies the same group reported that the offspring of rats with induced endometriosis exhibited altered gene expression in their own embryos thus providing a possible mechanism for the reported increase in incidence in families with endometriosis

415

(90). Some interesting data have also been reported in a rabbit model of autologous uterine transplantation which showed reduced fertility also most likely due to ovarian defects which they speculated might have been due to increased PGF concentrations in the peritoneal fluid, providing an interesting parallel with more recent rodent studies (91). Women with

420

endometriosis are reported to have oocytes that are more likely to fail *in vitro* maturation and to show altered morphology during IVF than women with other causes of infertility (92) and

in this regard it appears insights from animal models may be further advanced than those in women.

Practice Points

- Primates with induced endometriosis exhibit time-dependent changes in gene expression in their eutopic endometrium
- In rodent models induction of endometriosis results in changes in the constituents of the peritoneal fluid.
- Infertility in rodents with induced endometriosis has been associated with an adverse ovarian phenotype

7. Future Directions and Priorities

A number of models of endometriosis have been developed and their application has informed our understanding of genetic factors, regulatory molecules and mechanisms that underpin symptoms, including pain and infertility. There are clear advantages to using primates, such as the Baboon, in endometriosis research as they have menstrual cycles and their size allows for repeated sampling of eutopic endometrium and lesions. However, their use is very tightly regulated and testing of compounds restricted by the small numbers of animals that can be tested. Furthermore, whilst rodent models offer an attractive platform for testing of drugs that might treat pain or infertility, each model requires careful validation for its particular application – for example the widespread use of ovariectomy and oestrogen supplementation precludes studies related to infertility and may also compromise those related to pain mechanisms.

One topic not yet addressed by existing animal models is the impact of other conditions on women with endometriosis. A key example is adenomyosis (invasion of endometrial tissue

into the muscular wall of the uterus) with reports ranging from 20 to as high as 90% prevalence in women. A mouse model of adenomyosis based on treatment of neonatal females with oestrogens has been described (93) but apart from tests with GnRH antagonists there is limited overlap with studies in endometriosis models.

450

In many cases it is a combination of approaches using one or more animal models that may best inform the development of a new therapy or the repurposing of an existing drug. A recent example was published by Taylor and colleagues (94). They used the Baboon model of endometriosis to extend and validate studies in cells and mice to show that treatment with a
455 statin (Simvastatin) decreased the growth of endometrial lesions, modulated the expression of oestrogen receptors and reduced inflammation. Although this may suggest promise as a drug therapy, others have raised concerns about the impact such a treatment may have on fertility (95), which perfectly illustrates the challenges faced in moving from results in model systems to clinical trials. Furthermore, translation of pre-clinical models will likely require a stratified
460 approach as the human disease has a heterogeneous presentation and peritoneal, ovarian and deep infiltrating endometriosis may represent distinct disease aetiologies. Notably, the majority of current animal models recapitulate peritoneal disease and thus translation of therapies that show promise will require carefully designed clinical trials with targeted patient cohorts to ensure best chance of showing efficacy in humans. More work is needed to
465 improve modelling of ovarian and deep infiltrating endometriosis in animal systems to expand the range of pre-clinical targets and enhance the likelihood of developing new successful therapies.

Research Agenda

- 470
- Endometriosis is a complex disorder and no single animal model can fully recapitulate the human disorder.
 - Primates can develop natural or induced endometriosis but they are expensive to house, cannot be used for large group studies, require large amounts of drugs and it is not ethical to use them for routine screening.
- 475
- A widely used rat model develops peripheral and vaginal hyperplasia but lesions are induced using surgery with a cystic phenotype
 - Xenograft models are limited by the current need to use immune-incompetent recipients.
 - Studies on pain mechanisms are increasingly being reported but those focused on
- 480
- altered fertility are limited in number.
 - New models are needed to model co-morbidities
 - Development of new models is required to compare the impact(s) of peripheral, ovarian and deep disease on pain and/or infertility.

485 **Figure Legends.****Figure 1. Baboon model of endometriosis.**

Endometriosis can be experimentally induced in adult female olive baboons (*Papio anubis*) by i.p. inoculation of autologous menstrual endometrium on Day 2 of menses for two consecutive menstrual cycles. Under laparoscopic guidance, ~1 g of menstrual tissue and fluid is deposited at four sites: the pouch of Douglas, the uterine fundus, the cul de sac and the ovaries. Diagnostic laparoscopies accompanied by endometrectomies to harvest eutopic tissue are performed on Days 8–12 post-ovulation at 1, 3, 6, 9 and 12 months following the second inoculation. Image adapted from a paper published by the Fazleabas group in 2012 (27).

495 **Figure 2. Mouse model of Endometriosis.**

Diagram adapted from Greaves et al (44). In this model the ‘donor’ endometrial tissue was from a mouse that had been hormonally manipulated so that the endometrial tissue was subject to increased tissue degradation (e.g. MMP activation) that mimicked processes observed in menstrual tissue from women (52). Endometrial tissue was harvested 4 hours after removal of a progesterone pellet (active breakdown). Menstrual tissue was injected i.p. and lesions were recovered 21 days later. The histology of the lesions resembled those in women with overexpression of ER β and evidence of neuroangiogenesis (72).

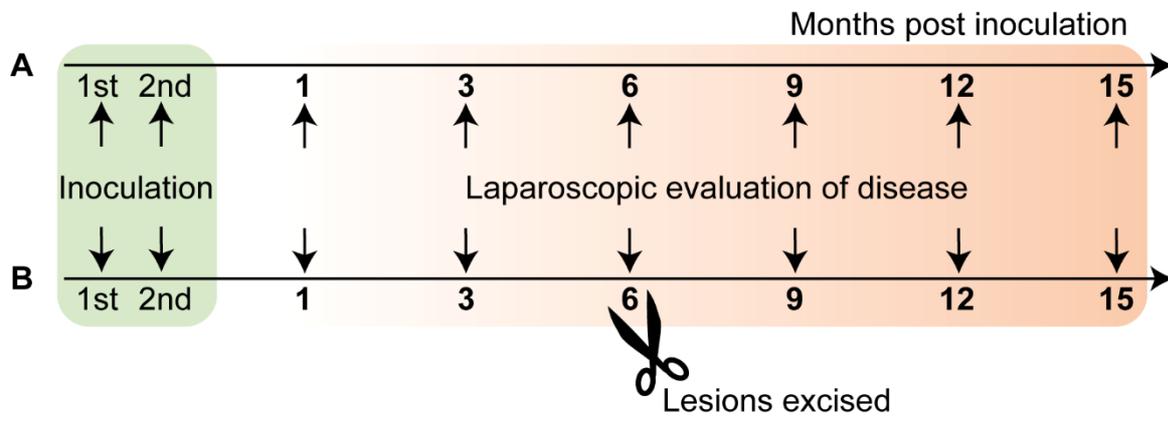
505 **Figure 3. Summary of the changes in gene expression detected in the eutopic endometrium of baboons with induced endometriosis.**

Endometrial samples recovered in between 1 and 15 months after induction of endometriosis were subjected to genomic analysis as described in (86). Results revealed dynamic temporal changes that could be grouped into early, transition or late. One of the key genes which changed was HOXA10 and its down regulation in late stage samples would be consistent with

510 observations that endometrium in women exhibits progesterone resistance (87). Image adapted from (25).

ACCEPTED MANUSCRIPT

Figure 1.

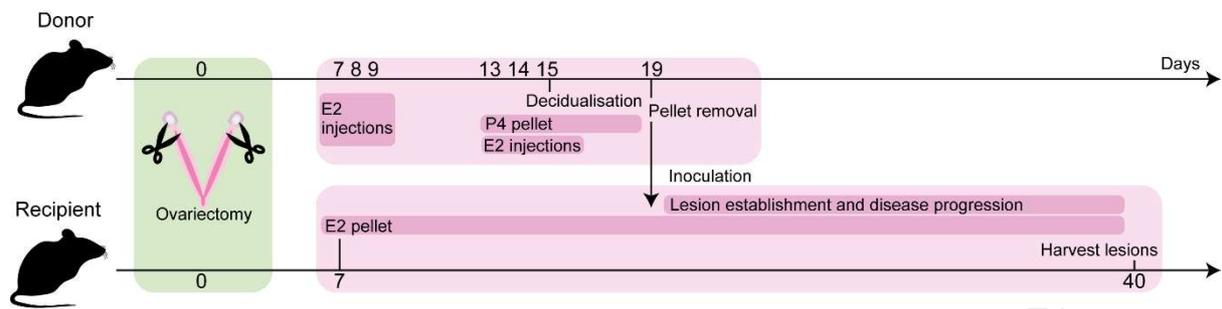


515

ACCEPTED MANUSCRIPT

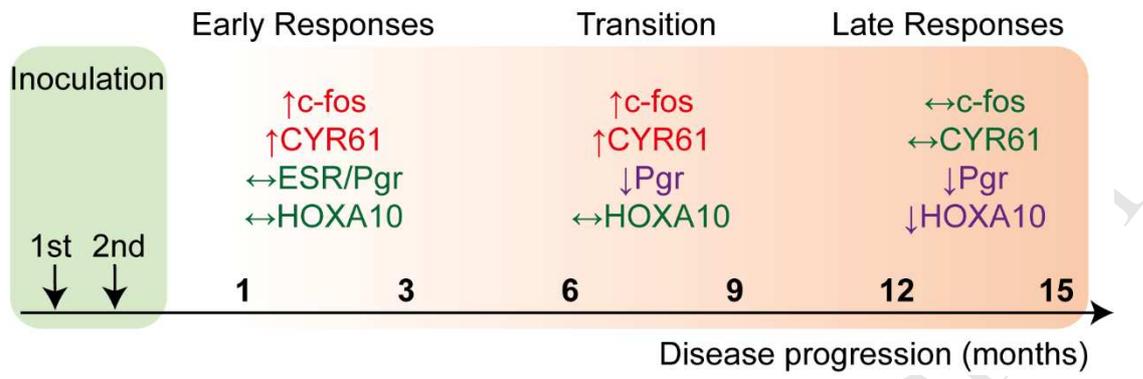
Figure 2.

520



ACCEPTED MANUSCRIPT

Figure 3.



References. * top ten

1. Bulun SE. Endometriosis. *N Engl J Med* 2009; 360:268-279
- 530 2. Arruda MS, Petta CA, Abrão MS, Benetti-Pinto CL. Time elapsed from onset of symptoms to diagnosis of endometriosis in a cohort study of Brazilian women. *Hum Reprod* 2003; 18:756-759
3. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril* 1997; 67:817-821
- 535 4. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG. Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. *Hum Reprod* 2007; 22:266-271
- 540 5. Vercellini P, Trespidi L, De Giorgi O, Cortesi I, Parazzini F, Crosignani PG. Endometriosis and pelvic pain: relation to disease stage and localization. *Fertil Steril* 1996; 65:299-304
6. *Stratton P, Berkley KJ. Chronic pelvic pain and endometriosis: translational evidence of the relationship and implications. *Hum Reprod Update* 2011; 17:327-346
- 545 7. Asante A, Taylor RN. Endometriosis: the role of neuroangiogenesis. *Annu Rev Physiol* 2011; 73:163-182
8. Marki G, Bokor A, Rigo J, Rigo A. Physical pain and emotion regulation as the main predictive factors of health-related quality of life in women living with endometriosis. *Hum Reprod* 2017; 32:1432-1438
- 550 9. Pallacks C, Hirchenhain J, Krussel JS, Fehm TN, Fehr D. Endometriosis doubles odds for miscarriage in patients undergoing IVF or ICSI. *Eur J Obstet Gynecol Reprod Biol* 2017; 213:33-38
10. Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. *Am J Pathol* 1927; 3:93-110.143
- 555 11. Brown J, Farquhar C. Endometriosis: an overview of Cochrane Reviews. *Cochrane Database Syst Rev* 2014; 3:CD009590
12. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril* 2012; 98:511-519
- 560 13. Young VJ, Brown JK, Saunders PT, Horne AW. The role of the peritoneum in the pathogenesis of endometriosis. *Hum Reprod Update* 2013; 19:558-569
14. Montgomery GW, Nyholt DR, Zhao ZZ, Treloar SA, Painter JN, Missmer SA, Kennedy SH, Zondervan KT. The search for genes contributing to endometriosis risk. *Hum Reprod Update* 2008; 14:447-457
- 565 15. Rogers PA, Adamson GD, Al-Jefout M, Becker CM, D'Hooghe TM, Dunselman GA, Fazleabas A, Giudice LC, Horne AW, Hull ML, Hummelshoj L, Missmer SA, Montgomery GW, Stratton P, Taylor RN, Rombauts L, Saunders PT, Vincent K, Zondervan KT, Endometriosis WWCfRPi. Research Priorities for Endometriosis. *Reprod Sci* 2017; 24:202-226
- 570 16. Horne AW, Saunders PTK, Abokhrais IM, Hogg L, Endometriosis Priority Setting Partnership Steering G. Top ten endometriosis research priorities in the UK and Ireland. *Lancet* 2017; 389:2191-2192

17. Greaves E, Critchley HOD, Horne AW, Saunders PTK. Relevant human tissue resources and laboratory models for use in endometriosis research. *Acta Obstet Gynecol Scand* 2017; 96:644-658
- 575 18. Grummer R. Animal models in endometriosis research. *Hum Reprod Update* 2006; 12:641-649
19. Story L, Kennedy S. Animal studies in endometriosis: a review. *ILAR J* 2004; 45:132-138
- 580 20. Ami Y, Suzaki Y, Goto N. Endometriosis in cynomolgus monkeys retired from breeding. *J Vet Med Sci* 1993; 55:7-11
21. Nishimoto-Kakiuchi A, Netsu S, Matsuo S, Hayashi S, Ito T, Okabayashi S, Yasmin L, Yuzawa K, Kondoh O, Kato A, Suzuki M, Konno R, Sankai T. Characteristics of histologically confirmed endometriosis in cynomolgus monkeys. *Hum Reprod* 2016; 31:2352-2359
- 585 22. Dehoux JP, Defrere S, Squifflet J, Donnez O, Polet R, Mestdagt M, Foidart JM, Van Langendonck A, Donnez J. Is the baboon model appropriate for endometriosis studies? *Fertil Steril* 2011; 96:728-733 e723
23. D'Hooghe TM, Debrock S, Kyama CM, Chai DC, Cuneo S, Hill JA, Mwenda JM. Baboon model for fundamental and preclinical research in endometriosis. *Gynecol Obstet Invest* 2004; 57:43-46
- 590 24. D'Hooghe TM, Kyama CM, Chai D, Fassbender A, Vodolazkaia A, Bokor A, Mwenda JM. Nonhuman primate models for translational research in endometriosis. *Reprod Sci* 2009; 16:152-161
25. *Fazleabas AT. A baboon model for inducing endometriosis. *Methods Mol Med* 2006; 121:95-99
- 595 26. Wang C, Mavrogianis PA, Fazleabas AT. Endometriosis is associated with progesterone resistance in the baboon (*Papio anubis*) oviduct: evidence based on the localization of oviductal glycoprotein 1 (OVGP1). *Biol Reprod* 2009; 80:272-278
- 600 27. Harirchian P, Gashaw I, Lipskind ST, Braundmeier AG, Hastings JM, Olson MR, Fazleabas AT. Lesion kinetics in a non-human primate model of endometriosis. *Hum Reprod* 2012; 27:2341-2351
28. Donnez O, Orellana R, Van Kerk O, Dehoux JP, Donnez J, Dolmans MM. Invasion process of induced deep nodular endometriosis in an experimental baboon model: similarities with collective cell migration? *Fertil Steril* 2015; 104:491-497 e492
- 605 29. Schenken RS, Asch RH, Williams RF, Hodgen GD. Etiology of infertility in monkeys with endometriosis: measurement of peritoneal fluid prostaglandins. *Am J Obstet Gynecol* 1984; 150:349-353
- 610 30. Einspanier A, Lieder K, Bruns A, Husen B, Thole H, Simon C. Induction of endometriosis in the marmoset monkey (*Callithrix jacchus*). *Mol Hum Reprod* 2006; 12:291-299
31. Defrere S, Squifflet J, Lousse JC, Van Langendonck A, Gonzalez-Ramos R, Foidart JM, Jerome C, Delattre L, Donnez J, Dehoux JP. Unsuccessful induction of endometriosis in female rhesus macaques (*Macaca mulatta*). *Gynecol Obstet Invest* 2008; 66:84-90
- 615 32. Vernon MW, Wilson EA. Studies on the surgical induction of endometriosis in the rat. *Fertil Steril* 1985; 44:684-694
- 620 33. Berkley KJ, Cason A, Jacobs H, Bradshaw H, Wood E. Vaginal hyperalgesia in a rat model of endometriosis. *Neurosci Lett* 2001; 306:185-188

34. *Berkley KJ, Dmitrieva N, Curtis KS, Papka RE. Innervation of ectopic endometrium in a rat model of endometriosis. *Proc Natl Acad Sci U S A* 2004; 101:11094-11098
- 625 35. Cason AM, Samuelsen CL, Berkley KJ. Estrous changes in vaginal nociception in a rat model of endometriosis. *Horm Behav* 2003; 44:123-131
36. Zhang G, Dmitrieva N, Liu Y, McGinty KA, Berkley KJ. Endometriosis as a neurovascular condition: estrous variations in innervation, vascularization, and growth factor content of ectopic endometrial cysts in the rat. *Am J Physiol Regul Integr Comp Physiol* 2008; 294:R162-171
- 630 37. Saltan G, Sutar I, Ozbilgin S, Ilhan M, Demirel MA, Oz BE, Keles H, Akkol EK. *Viburnum opulus* L.: A remedy for the treatment of endometriosis demonstrated by rat model of surgically-induced endometriosis. *Journal of ethnopharmacology* 2016; 193:450-455
- 635 38. Prodromidou A, Pergialiotis V, Pavlakis K, Korou LM, Frountzas M, Dimitroulis D, Vaos G, Perrea DN. A Novel Experimental Model of Colorectal Endometriosis. *Journal of investigative surgery : the official journal of the Academy of Surgical Research* 2017:1-7
39. Alvarez P, Chen X, Hendrich J, Irwin JC, Green PG, Giudice LC, Levine JD. Ectopic uterine tissue as a chronic pain generator. *Neuroscience* 2012; 225:269-282
- 640 40. Burns KA, Rodriguez KF, Hewitt SC, Janardhan KS, Young SL, Korach KS. Role of estrogen receptor signaling required for endometriosis-like lesion establishment in a mouse model. *Endocrinology* 2012; 153:3960-3971
41. Zhao Y, Chen Y, Kuang Y, Bagchi MK, Taylor RN, Katzenellenbogen JA, Katzenellenbogen BS. Multiple Beneficial Roles of Repressor of Estrogen Receptor Activity (REA) in Suppressing the Progression of Endometriosis. *Endocrinology* 2016; 157:900-912
- 645 42. Zhao Y, Gong P, Chen Y, Nwachukwu JC, Srinivasan S, Ko C, Bagchi MK, Taylor RN, Korach KS, Nettles KW, Katzenellenbogen JA, Katzenellenbogen BS. Dual suppression of estrogenic and inflammatory activities for targeting of endometriosis. *Sci Transl Med* 2015; 7:271ra279
- 650 43. Rakhila H, Girard K, Leboeuf M, Lemyre M, Akoum A. Macrophage migration inhibitory factor is involved in ectopic endometrial tissue growth and peritoneal-endometrial tissue interaction in vivo: a plausible link to endometriosis development. *PLoS One* 2014; 9:e110434
- 655 44. *Greaves E, Cousins FL, Murray A, Esnal-Zufiaurre A, Fassbender A, Horne AW, Saunders PT. A novel mouse model of endometriosis mimics human phenotype and reveals insights into the inflammatory contribution of shed endometrium. *Am J Pathol* 2014; 184:1930-1939
45. Becker CM, Wright RD, Satchi-Fainaro R, Funakoshi T, Folkman J, Kung AL, D'Amato RJ. A novel noninvasive model of endometriosis for monitoring the efficacy of antiangiogenic therapy. *Am J Pathol* 2006; 168:2074-2084
- 660 46. Wilkosz S, Pullen N, de-Giorgio-Miller A, Ireland G, Herrick S. Cellular exchange in an endometriosis-adhesion model using GFP transgenic mice. *Gynecol Obstet Invest* 2011; 72:90-97
- 665 47. Cummings AM, Metcalf JL. Induction of endometriosis in mice: a new model sensitive to estrogen. *Reprod Toxicol* 1995; 9:233-238
48. Yuan M, Li D, An M, Li Q, Zhang L, Wang G. Rediscovering peritoneal macrophages in a murine endometriosis model. *Hum Reprod* 2017; 32:94-102

- 670 49. Pelch KE, Sharpe-Timms KL, Nagel SC. Mouse model of surgically-induced endometriosis by auto-transplantation of uterine tissue. *Journal of visualized experiments : JoVE* 2012:e3396
50. Ferrero H, Buigues A, Martinez J, Simon C, Pellicer A, Gomez R. A novel homologous model for noninvasive monitoring of endometriosis progression. *Biol Reprod* 2017; 96:302-312
- 675 51. Hirata T, Osuga Y, Yoshino O, Hirota Y, Harada M, Takemura Y, Morimoto C, Koga K, Yano T, Tsutsumi O, Taketani Y. Development of an experimental model of endometriosis using mice that ubiquitously express green fluorescent protein. *Hum Reprod* 2005; 20:2092-2096
- 680 52. Cousins FL, Murray A, Esnal A, Gibson DA, Critchley HO, Saunders PT. Evidence from a mouse model that epithelial cell migration and mesenchymal-epithelial transition contribute to rapid restoration of uterine tissue integrity during menstruation. *PLoS One* 2014; 9:e86378
- 685 53. Peterse DP, Fassbender A, O DF, Vanhie A, Saunders P, Vriens J, Binda MM, D'Hooghe TM. Laparoscopic Surgery: A New Technique to Induce Endometriosis in a Mouse Model. *Reprod Sci* 2016; 23:1332-1339
54. Cheng CW, Licence D, Cook E, Luo F, Arends MJ, Smith SK, Print CG, Charnock-Jones DS. Activation of mutated K-ras in donor endometrial epithelium and stroma promotes lesion growth in an intact immunocompetent murine model of endometriosis. *J Pathol* 2011; 224:261-269
- 690 55. Jafarabadi M, Salehnia M, Sadafi R. Evaluation of two endometriosis models by transplantation of human endometrial tissue fragments and human endometrial mesenchymal cells. *International journal of reproductive biomedicine* 2017; 15:21-32
- 695 56. Banu SK, Starzinski-Powitz A, Speights VO, Burghardt RC, Arosh JA. Induction of peritoneal endometriosis in nude mice with use of human immortalized endometriosis epithelial and stromal cells: a potential experimental tool to study molecular pathogenesis of endometriosis in humans. *Fertil Steril* 2009; 91:2199-2209
- 700 57. Hull ML, Escareno CR, Godsland JM, Doig JR, Johnson CM, Phillips SC, Smith SK, Tavare S, Print CG, Charnock-Jones DS. Endometrial-peritoneal interactions during endometriotic lesion establishment. *Am J Pathol* 2008; 173:700-715
58. *Hull ML, Johan MZ, Hodge WL, Robertson SA, Ingman WV. Host-derived TGFB1 deficiency suppresses lesion development in a mouse model of endometriosis. *Am J Pathol* 2012; 180:880-887
- 705 59. Hull ML, Prentice A, Wang DY, Butt RP, Phillips SC, Smith SK, Charnock-Jones DS. Nimesulide, a COX-2 inhibitor, does not reduce lesion size or number in a nude mouse model of endometriosis. *Hum Reprod* 2005; 20:350-358
- 710 60. Alvarez Gonzalez ML, Frankenne F, Galant C, Marbaix E, Foidart JM, Nisolle M, Beliard A. Mixed origin of neovascularization of human endometrial grafts in immunodeficient mouse models. *Hum Reprod* 2009; 24:2217-2224
61. Ahmad SF, Akoum A, Horne AW. Selective modulation of the prostaglandin F2alpha pathway markedly impacts on endometriosis progression in a xenograft mouse model. *Mol Hum Reprod* 2015; 21:905-916
- 715 62. Arosh JA, Lee J, Balasubramanian D, Stanley JA, Long CR, Meagher MW, Osteen KG, Bruner-Tran KL, Burghardt RC, Starzinski-Powitz A, Banu SK. Molecular and preclinical basis to inhibit PGE2 receptors EP2 and EP4 as a novel nonsteroidal therapy for endometriosis. *Proc Natl Acad Sci U S A* 2015; 112:9716-9721

- 720 63. *Braundmeier A, Jackson K, Hastings J, Koehler J, Nowak R, Fazleabas A. Induction of endometriosis alters the peripheral and endometrial regulatory T cell population in the non-human primate. *Hum Reprod* 2012; 27:1712-1722
64. Tanaka Y, Mori T, Ito F, Koshiba A, Takaoka O, Kataoka H, Maeda E, Okimura H, Mori T, Kitawaki J. Exacerbation of Endometriosis Due To Regulatory T-Cell Dysfunction. *J Clin Endocrinol Metab* 2017; 102:3206-3217
- 725 65. Lin YJ, Lai MD, Lei HY, Wing LY. Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. *Endocrinology* 2006; 147:1278-1286
66. Bacci M, Capobianco A, Monno A, Cottone L, Di Puppò F, Camisa B, Mariani M, Brignole C, Ponzoni M, Ferrari S, Panina-Bordignon P, Manfredi AA, Rovere-Querini P. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. *Am J Pathol* 2009; 175:547-556
- 730 67. Azuma Y, Taniguchi F, Nakamura K, Nagira K, Khine YM, Kiyama T, Uegaki T, Izawa M, Harada T. Lipopolysaccharide promotes the development of murine endometriosis-like lesions via the nuclear factor-kappa B pathway. *Am J Reprod Immunol* 2017; 77
- 735 68. Du Y, Liu X, Guo SW. Platelets impair natural killer cell reactivity and function in endometriosis through multiple mechanisms. *Hum Reprod* 2017; 32:794-810
69. Somigliana E, Vignani P, Zingrillo B, Ranieri S, Filardo P, Candiani M, Vignali M. Induction of endometriosis in the mouse inhibits spleen leukocyte function. *Acta Obstet Gynecol Scand* 2001; 80:200-205
- 740 70. *Huhtinen K, Stahle M, Perheentupa A, Poutanen M. Estrogen biosynthesis and signaling in endometriosis. *Mol Cell Endocrinol* 2012; 358:146-154
71. Fazleabas AT, Brudney A, Chai D, Langoi D, Bulun SE. Steroid receptor and aromatase expression in baboon endometriotic lesions. *Fertil Steril* 2003; 80 Suppl 2:820-827
- 745 72. Greaves E, Temp J, Esnal-Zufiurre A, Mechsner S, Horne AW, Saunders PT. Estradiol Is a Critical Mediator of Macrophage-Nerve Cross Talk in Peritoneal Endometriosis. *Am J Pathol* 2015; 185:2286-2297
73. Greaves E, Collins F, Esnal-Zufiurre A, Giakoumelou S, Horne AW, Saunders PT. Estrogen Receptor (ER) Agonists Differentially Regulate Neuroangiogenesis in Peritoneal Endometriosis via the Repellent Factor SLIT3. *Endocrinology* 2014; 155:4015-4026
- 750 74. McAllister SL, Dmitrieva N, Berkley KJ. Sprouted innervation into uterine transplants contributes to the development of hyperalgesia in a rat model of endometriosis. *PLoS One* 2012; 7:e31758
- 755 75. Tappe-Theodor A, Kuner R. Studying ongoing and spontaneous pain in rodents--challenges and opportunities. *Eur J Neurosci* 2014; 39:1881-1890
76. *Berkley KJ, McAllister SL, Accius BE, Winnard KP. Endometriosis-induced vaginal hyperalgesia in the rat: effect of estropause, ovariectomy, and estradiol replacement. *Pain* 2007; 132 Suppl 1:S150-159
- 760 77. Dmitrieva N, Faircloth EK, Pyatok S, Sacher F, Patchev V. Telemetric assessment of referred vaginal hyperalgesia and the effect of indomethacin in a rat model of endometriosis. *Frontiers in pharmacology* 2012; 3:158
- 765 78. Dmitrieva N, Nagabukuro H, Resuehr D, Zhang G, McAllister SL, McGinty KA, Mackie K, Berkley KJ. Endocannabinoid involvement in endometriosis. *Pain* 2010; 151:703-710

79. Dmitrieva N, Suess G, Shirley R. Resolvins RvD1 and 17(R)-RvD1 alleviate signs of inflammation in a rat model of endometriosis. *Fertil Steril* 2014; 102:1191-1196
- 770 80. Hernandez S, Cruz ML, Seguinot, II, Torres-Reveron A, Appleyard CB. Impact of Psychological Stress on Pain Perception in an Animal Model of Endometriosis. *Reprod Sci* 2017; 24:1371-1381
81. Alvarez P, Giudice LC, Levine JD. Impact of surgical excision of lesions on pain in a rat model of endometriosis. *European journal of pain* 2014;
- 775 82. Alvarez P, Bogen O, Chen X, Giudice LC, Levine JD. Ectopic endometrium-derived leptin produces estrogen-dependent chronic pain in a rat model of endometriosis. *Neuroscience* 2014; 258:111-120
83. *Greaves E, Horne AW, Jerina H, Mikolajczak M, Hilferty L, Mitchell R, Fleetwood-Walker SM, Saunders PT. EP2 receptor antagonism reduces peripheral and central hyperalgesia in a preclinical mouse model of endometriosis. *Scientific reports* 2017; 7:44169
- 780 84. McAllister SL, Giourgas BK, Faircloth EK, Leishman E, Bradshaw HB, Gross ER. Prostaglandin levels, vaginal innervation, and cyst innervation as peripheral contributors to endometriosis-associated vaginal hyperalgesia in rodents. *Mol Cell Endocrinol* 2016; 437:120-129
- 785 85. Practice Committee of the American Society for Reproductive M. Endometriosis and infertility: a committee opinion. *Fertil Steril* 2012; 98:591-598
86. *Afshar Y, Hastings J, Roqueiro D, Jeong JW, Giudice LC, Fazleabas AT. Changes in eutopic endometrial gene expression during the progression of experimental endometriosis in the baboon, *Papio anubis*. *Biol Reprod* 2013; 88:44
- 790 87. Kim JJ, Taylor HS, Lu Z, Ladhani O, Hastings JM, Jackson KS, Wu Y, Guo SW, Fazleabas AT. Altered expression of HOXA10 in endometriosis: potential role in decidualization. *Mol Hum Reprod* 2007; 13:323-332
88. Bilotas MA, Olivares CN, Ricci AG, Baston JI, Bengochea TS, Meresman GF, Baranao RI. Interplay between Endometriosis and Pregnancy in a Mouse Model. *PLoS One* 2015; 10:e0124900
- 795 89. Stille JA, Woods-Marshall R, Sutovsky M, Sutovsky P, Sharpe-Timms KL. Reduced fecundity in female rats with surgically induced endometriosis and in their daughters: a potential role for tissue inhibitors of metalloproteinase 1. *Biol Reprod* 2009; 80:649-656
- 800 90. *Birt JA, Taylor KH, Davis JW, Sharpe-Timms KL. Developmental exposure of fetal ovaries and fetal germ cells to endometriosis in an endometriosis model causes differential gene expression in the preimplantation embryos of the first-generation and second-generation embryos. *Fertil Steril* 2013; 100:1436-1443
- 805 91. Schenken RS, Asch RH. Surgical induction of endometriosis in the rabbit: effects on fertility and concentrations of peritoneal fluid prostaglandins. *Fertil Steril* 1980; 34:581-587
92. Sanchez AM, Vanni VS, Bartiromo L, Papaleo E, Zilberberg E, Candiani M, Orvieto R, Vignano P. Is the oocyte quality affected by endometriosis? A review of the literature. *J Ovarian Res* 2017; 10:43
- 810 93. Greaves P, White IN. Experimental adenomyosis. *Best Pract Res Clin Obstet Gynaecol* 2006; 20:503-510
94. Taylor HS, Alderman Iii M, D'Hooghe TM, Fazleabas AT, Duleba AJ. Effect of simvastatin on baboon endometriosis. *Biol Reprod* 2017; 97:32-38
- 815 95. Vitagliano A, Noventa M, Quaranta M, Gizzo S. Statins as Targeted "Magical Pills" for the Conservative Treatment of Endometriosis: May Potential Adverse Effects

on Female Fertility Represent the "Dark Side of the Same Coin"? A Systematic Review of Literature. *Reprod Sci* 2016; 23:415-428

ACCEPTED MANUSCRIPT