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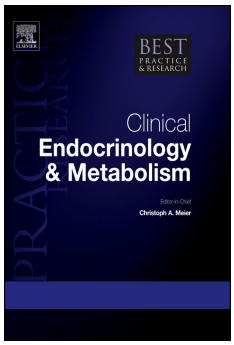

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Animal Models of Endometriosis: replicating the aetiology and symptoms of the human disorder.

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Abstract

Endometriosis is a chronic incurable disorder that affects 1 in 10 women of reproductive age: 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 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.

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

General Practice Points

- 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.
- 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 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.

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

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 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 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 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 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

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 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 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 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 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

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). 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).

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 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 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.

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 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 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 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 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

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 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 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 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 prostaglandins (e.g. PGF2α, 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 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 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 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 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 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-β1, 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-β1 in the resultant 'lesions' (68).

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 ERß 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.

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

studies they reported the macrophages in lesions were ERß-positive (72) and that treatment of mice with induced endometriosis with the ERß-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 ERαKO 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 co-ordinated 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

- 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.
- 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.

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 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 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, Herandez 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.

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. 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 (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 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 endometriuim
- 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.

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 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 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 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

- 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.
- 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 altered fertility are limited in number.
- New models are needed to model co-morbitities
- Development of new models is required to compare the impact(s) of peripheral, ovarian and deep disease on pain and/or infertility.

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).

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 degredation (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 ERß and evidence of neuroangiogenesis (72).

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

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

Figure 1.

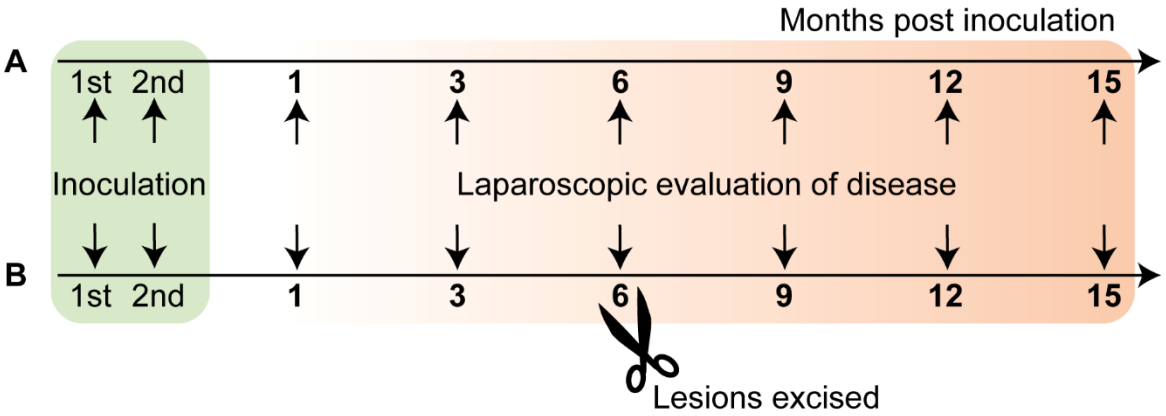

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Figure 2.

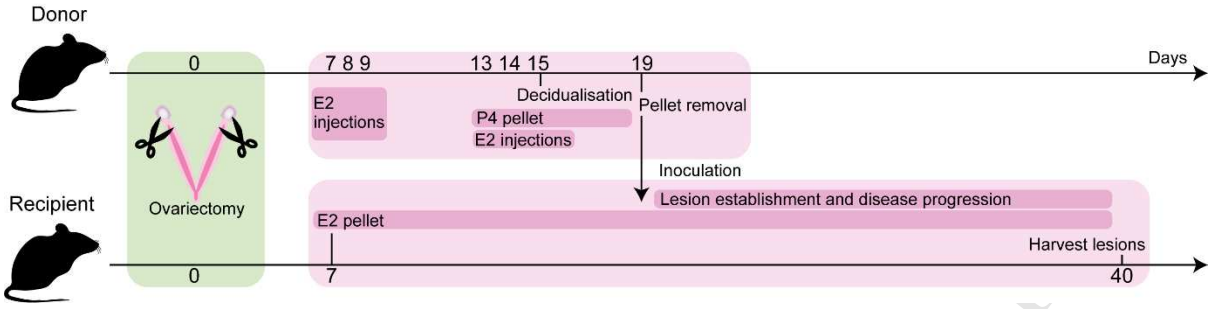
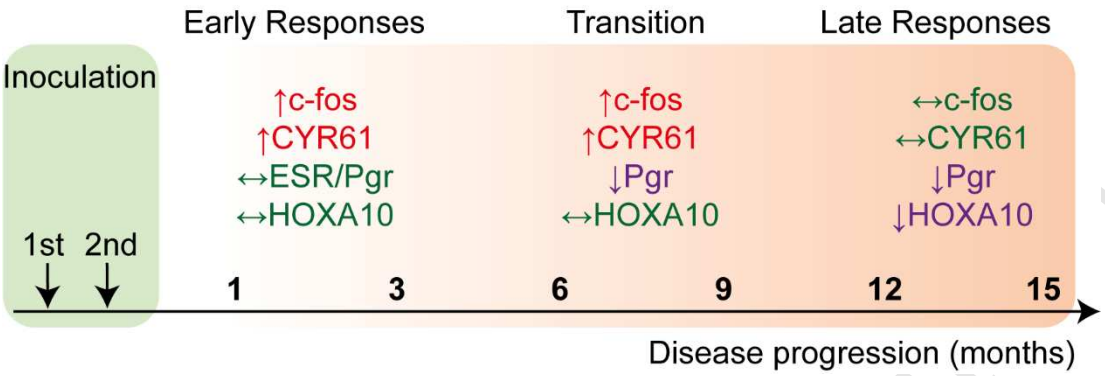


Figure 3.



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