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Rationale for using surrogate markers for adenomyosis lesions in animal models: A narrative review

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ABSTRACT

Adenomyosis is a gynecological disease characterized by the invasion of endometrial glands and stroma into the myometrium, often causing symptoms of dysmenorrhea, abnormal uterine bleeding, and infertility. Basic research using animal models is important for understanding the pathogenesis mechanism and evaluating new interventions, but clinical symptoms in humans are difficult to measure directly in experimental animals. Therefore, surrogate markers that can objectively represent the clinical condition are needed. Methods: This literature review was compiled following the PRISMA 2020 guidelines. Articles were searched in PubMed, Scopus, and Web of Science databases for publications from 2015–2025. Inclusion criteria included original research articles (controlled trials, cohort, or cross-sectional studies) written in English, focusing on the use of surrogate markers in animal models of adenomyosis. Review articles, editorials, and publications without DOI were excluded. Results: A total of six primary studies met the inclusion criteria, most of which used the Institute of Cancer Research (ICR) mouse model with neonatal tamoxifen induction. Surrogate markers used can be grouped into three categories: (1) histological markers, such as the depth of myometrial infiltration and the degree of fibrosis (Masson staining, collagen I/IV, α-SMA), (2) functional markers, such as uterine contractility and pain behavior tests (hotplate latency), and (3) molecular markers, such as TGF-β1/p-Smad3, COX-2, TRPV1, NGF, PR-B, OTR, vimentin, and E-cadherin. In addition, several studies have assessed reproductive markers (LIF expression and implantation rate) as fertility surrogates. The results of these studies show a consistent relationship between adenomyosis progression and fibrosis, molecular dysregulation, hyperalgesia, and changes in uterine contractility. Interventions such as resveratrol, anti-platelet, and TGF-β1 neutralizing antibodies have been shown to improve these markers. Surrogate markers in animal models of adenomyosis have proven rational and useful for assessing disease progression and the effects of interventions. Although translational validity to humans is still limited, histological, functional, molecular, and reproductive markers can be an important bridge between basic research and clinical application in adenomyosis.

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INTRODUCTION

Adenomyosis is a common gynecological condition. Uterine adenomyosis is characterized by the presence of endometrial glands and stroma within the myometrium, which is usually associated with pelvic pain, abnormal uterine bleeding (AUB), or infertility (Putra & Anggraini, 2022). Poor quality of life due to delayed diagnosis is a major problem in the treatment of both disorders. The presence of ectopic endometrial glands and stroma is associated with inflammation, fibrosis, and aberrant angiogenesis (Benagiano, Brosens, & Habiba, 2014; Bulun, 2022; Maruyama, Imanaka, Nagayasu, Kimura, & Kobayashi, 2020). The prevalence of adenomyosis ranges from 5-70% with more recent data suggesting 20-35% (Abbott, 2017; Lessey & Young, 2019; Struble, Reid, & Bedaiwy, 2016).

Adenomyosis is described as a disease in pre-menopausal and multiparous women in their thirties to forties (Abbott, 2017; Lessey & Young, 2019; Struble et al., 2016). Basic research using animal models plays a crucial role in elucidating molecular mechanisms and assessing the potential for new interventions for adenomyosis. However, a major challenge in this research is the limited ability to directly measure human clinical symptoms in experimental animals. Surrogate markers that can be objectively measured and represent human clinical conditions are needed to address this issue (Andriyani et al., 2024),(Agustiningsih, 2025).

These markers can be histological changes, functional markers, or molecular markers (Andriyani et al., 2024),(Wahyuhadi, 2020). Several studies have also assessed reproductive outcomes such as LIF expression and implantation rates as a fertility surrogate. This review aims to summarize the current evidence regarding the use of surrogate markers in animal models of adenomyosis, assess the rationale for their use, and identify opportunities and limitations in applying animal research findings to human clinical contexts.

RESEARCH METHOD

This article was compiled following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines, ensuring that the article complies with these standards. This is to ensure the accuracy of the findings from the included studies. The purpose of this literature review is to demonstrate the rationale for using surrogate markers for adenomyosis lesions in animal models. Included studies must meet the following criteria: 1) written in English, 2) published after 2015, and 3) be in the form of a cross-sectional study, cohort study, or clinical trial.

Editorials, scientific journals without DOIs, and review articles were excluded from the study. The study search was conducted using Google Scholar, PubMed, and SagePub databases, as well as other relevant databases. We determined whether a journal met the inclusion criteria after reviewing the abstract and title of each study. Authors determined which previous studies would serve as sources for the articles and selected those studies. All included journals must be written in English and unpublished. Only publications meeting all inclusion criteria were considered for this narrative review.

Table 1. Search results

Writer	Method	Number of samples (animals)	Marker	Main output
Shen et al.,	ICR mouse,	28 (induced) vs	TGF-β1, p-Smad3,	Depth of myometrial infiltration,

Writer	Method	Number of samples (animals)	Marker	Main output
2016(Shen, Liu, Zhang, & Guo, 2016)	neonatal (tamoxifen)-induced adenomyosis; histological descriptive study	32 (control)	vimentin, E-cadherin, α-SMA, PR-B; platelet aggregation	increase in TGF-β1/p-Smad3 with progression; evidence of EMT & fibrogenesis as molecular markers related to fibrogenesis.
Zhu et al, 2015(Zhu, Chen, Zhang, Liu, & Guo, 2015)	Mouse ICR, tamoxifen-induced; non-hormonal interventions (resveratrol [RSV])	28 (induced) vs 12 (control); then randomization to treatment group	Hotplate latency (pain test), uterine contractility, fibrosis (Masson), protein: COX-2, TRPV1, GAD65 (brain)	RSV decreases infiltration depth, increases hotplate latency (reduces hyperalgesia), decreases uterine contractility and fibrosis.
Zhu et al. 2016(Zhu, Chen, Shen, Liu, & Guo, 2016)	Mouse ICR, tamoxifen-induced; randomization to ozagrel/platelet depletion	57 (induced) + 12 (control)	*Platelet aggregation*(CD41), hotplate latency, uterine contractility, collagen I/IV, PR-B, COX-2, TRPV1, and OTR	Anti-platelet (ozagrel/depletion) suppresses myometrial infiltration, increases hotplate latency (reduces pain), reduces contractility and fibrosis supporting the role of the platelet/TGF pathway.
Kay et al., 2020(Kay et al., 2020)	ICR mouse; tamoxifen model; intrauterine injection of anti-TGF-β1 neutralizing antibody (intervention)	4 mouse models	TGF-β1 (target), Leukemia inhibitory factor (LIF) as a marker of receptivity; collagen (Masson)	Net effect: TGF-β1 neutralization → reduces fibrosis, increases LIF expression, improves implantation rate / pregnancy outcome (surrogate fertility).
Li et al. 2020(Li, Zhang, Jin, Xia, & Zhang, 2021)	Adenomyosis mouse model; proteomic and IHC analysis	20 (induced) + 20 (control)	NGF & its receptors (neurotrophic markers), protein-profiling (proteomics)	Increased NGF and neurogenic proteins in the uterus and DRG are consistent with disease progression → NGF as a marker of neuro-angiogenesis/pain.
Qu et al 2021(Qu, Lu, Bellve, Lifshitz, & ZhuGe, 2021)	Tamoxifen rat model; measurement of uterine contractile activity and Ca^{2+} oscillation	6 mice	Uterine peristalsis / Ca^{2+} oscillation (physiologic functional marker), OTR expression	Changes in Ca^{2+} oscillation & hyperperistalsis are associated with adenomyosis; these functional markers are used as surrogates for dysmenorrhea/dysperistalsis.

RESULTS AND DISCUSSIONS

The depth of myometrial infiltration and the degree of fibrosis (assessed by Masson or Van Gieson staining, as well as collagen I/IV and α-SMA markers) can be the most frequently used histological surrogates Shen et al. (2016) and Zhu et al. (2015, 2016) confirmed that adenomyosis progression is associated with increased fibrosis. This is in line with the activation of the TGF-β1/p-Smad3 pathway and the occurrence of epithelial-mesenchymal transition (EMT) and fibroblast-mesenchymal transition (FMT). This fibrosis is seen as a structural representation that can surrogate the clinical parameters of abnormal bleeding or chronic pain in patients (Abbott JA, 2017).

Several studies have evaluated uterine contractility in vitro and ex vivo as a surrogate marker for functional dysmenorrhea. Decreased contraction amplitude and frequency following therapy (e.g., resveratrol or antiplatelet therapy) suggest the potential of this surrogate in assessing intervention effectiveness. Behavioral pain tests, such as hotplate latency, can be used to assess hyperalgesia. Consistent results from several studies indicate that therapy that suppresses lesion progression can also increase the pain threshold. This makes hotplate latency a fairly representative surrogate for visceral pain in humans (Zhu et al., 2016, 2015).

Molecular studies have shown that several markers, such as TGF-β1, p-Smad3, COX-2, TRPV1, NGF, PR-B, OTR, vimentin, and E-cadherin, act as surrogate indicators for certain pathophysiological processes. TGF-β1 and p-Smad3 expression reflects the degree of fibrogenesis. Increased NGF is associated with neuroangiogenesis and pain hypersensitivity. A positive response to interventions on these markers strengthens their validity as relevant surrogates. Furthermore, reproductive outcomes are evaluated through Leukemia Inhibitory Factor (LIF) expression and implantation rates or successful pregnancies. Neutralization of TGF-β1 in animal models has been shown to reduce fibrosis, increase LIF expression, and improve pregnancy outcomes. This could be considered as a fertility surrogate in adenomyosis.

Overall, several studies have shown that the use of surrogate markers in adenomyosis animal models is divided into three large groups, including: (1) histological markers that assess changes in tissue structure, (2) functional markers that reflect pain and uterine contractility, and (3) molecular markers that describe the main biological mechanisms. These three groups of markers are relatively consistent in showing the relationship between adenomyosis progression and the degree of infiltration, fibrosis, hyperalgesia, and molecular dysregulation

Discussion

This review demonstrates that the use of surrogate markers in animal models of adenomyosis is a rational and necessary approach. Major human clinical manifestations, such as dysmenorrhea, menorrhagia, and infertility, cannot be directly measured in animal models. Surrogate markers found in the literature can be grouped into histological, functional, and molecular markers, each with its own advantages and limitations. Histological markers, such as the depth of myometrial infiltration and the degree of fibrosis, are the most frequently used surrogates (Aisa MC, Cappuccini B, Barbati A, & et al, 2016; Tellum & Munro, 2022).

Histological markers are easily measured objectively through histopathology and have a strong correlation with disease progression. Fibrosis is commonly associated with abnormal bleeding and chronic pain. Measurable infiltration and fibrosis in animals do not always reflect the severity of symptoms in humans, given the subjective, psychosocial, and hormonal factors that influence the patient's clinical experience. Functional markers such as uterine contractility and pain behavior (e.g., hotplate latency) offer a closer approximation to clinical symptoms (Struble J, Reid S, & Bedaiwy MA, 2016). Decreased contractility or increased pain threshold after intervention supports its use as a surrogate for dysmenorrhea. However, interpreting pain behavior in animals is not entirely equivalent to human pain, which is more complex and multidimensional (Zhang L, Rao F, & Setzen R, 2017)

Molecular markers provide mechanistic insights into pathogenesis, such as TGF-β1/p-Smad3 in fibrosis, NGF in pain, and COX-2 and TRPV1 in inflammation. The use of these markers strengthens the biological basis of histological and functional changes. However, a major challenge is translational validity, as species differences can lead to differences in the expression of certain molecules compared to humans. Several studies have explored reproductive markers, such as LIF expression and implantation success, which may serve as fertility surrogates. Neutralization of TGF-β1 improves pregnancy outcomes in animal models, supporting the relevance of these markers for interventional research on infertility in adenomyosis (Kay et al., 2020),(Biomedik & Raharja, nd).

Surrogate markers offer the advantages of objective measurement, high reproducibility, and ease of intervention evaluation. However, they are not without several limitations (Ardiana, 2025),(Pagala, 2020). First, no single marker can represent the entire spectrum of clinical manifestations of adenomyosis. Second, most studies used ICR mice with neonatal tamoxifen induction, which, while consistent, does not necessarily reflect the heterogeneity of adenomyosis in humans. Third, some molecular markers still require further validation for their clinical correlation in patients (Agustin, 2022).

Results from animal model studies provide an important foundation for understanding the underlying mechanisms of adenomyosis and evaluating experimental therapies. However, interpretation of the effectiveness of interventions should be approached with caution. Clinical trials are still needed to confirm whether changes in surrogate markers in animals are truly associated with improvements in symptoms and quality of life in human patients. This confirms that the use of surrogate markers in animal models of adenomyosis is a strategic step in bridging the gap between basic research and clinical application.

CONCLUSION

The use of surrogate markers in animal models of adenomyosis is a rational approach to assess disease progression and the effects of interventions. Histological, functional, molecular, and reproductive markers have been shown to consistently reflect pathological changes, although translational validity to humans remains limited. These surrogate markers are important as a bridge between basic research and clinical applications in adenomyosis.

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