

Prenatal Origins of Endometriosis Pathology and Pain: Reviewing the Evidence of a Role for Low Testosterone

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Abstract: Endometriosis is a polygenic, estrogen-dependent, inflammatory disorder of uncertain aetiology associated with pain, infertility and reduced quality of life. While the positive association between endometriosis and estrogen is established, a suite of recent studies has demonstrated an inverse association between the presence of endometriosis lesions and levels of testosterone both prenatally and postnatally. The following narrative review provides new insights into the roles of testosterone in the aetiology, diagnosis, and management of endometriosis and associated symptoms, especially pain. A relatively short anogenital distance (AGD) is indicative of lower levels of testosterone during fetal development. A shorter AGD has recently been correlated with both a higher risk of developing endometriosis in adult life, and with known correlates of endometriosis including earlier onset of reproductive cycling, lower ovarian follicle number, lower postnatal testosterone, and premature ovarian insufficiency. During adult life, lower levels of testosterone are positively associated with key comorbidities of endometriosis, including days per month of pelvic pain and increased pain sensitivity. Biochemically, lower levels of testosterone are associated with higher levels of pro-inflammatory IL-1 β and lower levels of β -endorphin. In rodents, prenatal administration of testosterone to females reduces their pain sensitivity in adulthood. The emerging convergent links of endometriosis with low prenatal and postnatal testosterone provide evidence of a centrally mediated effect beginning in early prenatal development, and persisting through adult life, with notable effects on pain sensitivity. They generate a novel conceptual framework for understanding, studying and treating this disorder, whereby endometriosis is mediated by a combination of high estrogen in endometrial tissue with low systemic and ovarian testosterone.

Keywords: endometriosis, pain, testosterone, anogenital distance, fetal development

Lay Summary

Endometriosis is a painful reproductive disorder, associated with reduced fertility, that affects 5–15% of women of reproductive age. Its causes are largely unknown, although the important roles of hormones, inflammation and cytokines are recognized. Recent studies indicate that a person's lifetime risk of developing endometriosis is associated with low levels of testosterone during fetal development. Low levels of testosterone before birth are associated with lower levels of testosterone in adult life, and lower testosterone as an adult is associated with increased days per month of pelvic pain. Optimal levels of testosterone are important for many aspects of female reproduction, including egg development, ovulation, fertilization, and embryo implantation. The finding that low testosterone is associated with an increased risk for endometriosis in adults has important implications. New therapies that normalize testosterone activity without undue side effects may offer ways to reduce the incidence and symptoms of endometriosis in women.

Introduction

Endometriosis is a gynaecological disorder affecting about 10% of women globally that is associated with inflammation and activation of the immune system.^{1–3} Endometriosis is characterized by the presence of endometrial glands and stroma

outside the uterus, most commonly involving the ovaries, pelvic peritoneum, or rectovaginal area.² It is associated with the presence of pelvic pain and a diminished quality of life in those affected.⁴ The altered immune environment present within the peritoneal cavity of women with endometriosis includes macrophage activation with increased release of proinflammatory cytokines including interleukin 1, nuclear factor kappa B (NF- κ B) and tumor necrosis factor alpha (TNF α).^{3,5} In most cases, a definitive diagnosis of endometriosis requires visualization of lesions during a laparoscopic surgical procedure, with its associated surgical risks and health economic burden.⁶

Traditionally, endometriosis has been considered as a disorder predominantly driven by estrogen, while androgens have been regarded as hormones mainly relevant to the physiology of males. However, androgen receptors are also widely distributed in female tissues including the uterus, breasts, endometrium, ovary, brain, bone, and muscle. As such, androgens play essential roles in female reproduction. In addition, androgen levels modulate symptoms of mental and physical well-being including cognitive performance, cardiovascular health and sexual function.^{7–10} This is established for males but relatively under-researched in females.¹⁰

A suite of recent studies has provided novel evidence pointing to low prenatal and postnatal levels of testosterone as contributing factors in the development of endometriosis lesions and many of the diverse traits and symptoms linked to the condition, including its cardinal symptom, pain. In this article, we review and describe this new evidence, explain how it provides a novel and productive framework for understanding the etiology of endometriosis, and discuss implications of these findings for treatment.

Methods

This article provides a hypothesis-driven narrative review describing and evaluating the theory that low testosterone plays key roles in endometriosis risks and symptoms, especially pain. Literature for the review was collected through comprehensive searches that focused on the diverse roles of prenatal and postnatal testosterone in endometriosis and its correlates. Articles were included if they provided insight into the hypothesis that testosterone mediates endometriosis risks and symptoms.

AGD as a Marker for Prenatal Testosterone Effects on Endometriosis Risk

Testosterone regulates sex differentiation and divergence in early human prenatal development.¹¹ The anogenital distance (AGD), measured either from the anus to the posterior fourchette (AGD-AF) or the anus to the clitoral surface (AGD-AC) (Figure 1),¹² provides a convenient, easily measurable proxy for androgen levels present during the early “programming window” of fetal development in both sexes. Perineal growth at this time is caused by androgen-mediated caudal movement of the genital tubercle.¹² Higher androgen levels result in greater AGD length, and lower levels result in shorter AGD length. This finding is consistent with the approximately twofold longer AGD in males compared to females.¹³

Increasing evidence over recent years, from both animal and human studies, demonstrates that a relatively short AGD is strongly associated with the presence of endometriosis and its clinical correlates. In humans across several study populations, AGD-AC, AGD-AF, or both were significantly shorter among women with endometriosis than among matched controls.^{14–19} These AGD differences are substantial and in some circumstances predictive; for example, Mendiola et al¹⁴ computed an odds ratio of 41.6 ($p = 0.002$) for deep infiltrating endometriosis in women with AGD below the mean, compared to women with AGD above the mean. Crestani et al¹⁸ reported, for endometriosis as a whole, a diagnostic specificity of 0.98 and a positive predictive value of 0.97, for an MRI-measured AGD-AF length of less than 20-mm. By contrast, a recent study²⁰ found a lack of significant difference, perhaps in part because sample sizes were relatively small. These studies are summarized in the systemic review of AGD and its relationship to endometriosis and polycystic ovarian syndrome by Pan et al.²¹

A shorter AGD has also been associated with several correlates of endometriosis, including low relative fertility. These correlates include lower levels of anti-Müllerian hormone (AMH) in subjects without endometriosis undergoing in vitro fertility treatment²² and the presence of premature ovarian insufficiency.²³ Moreover, among 100 typical, college-aged premenopausal women, shorter AGD has been associated with lower serum testosterone,²⁴ lower ovarian follicle number,²⁵ and more-regular menstrual cycles in their mothers.²⁶ These studies raise the possibility of phenotypic consequences of low fetal testosterone beyond the presence or absence of endometriosis lesions.

Two methods for measuring anogenital distance (AGD)

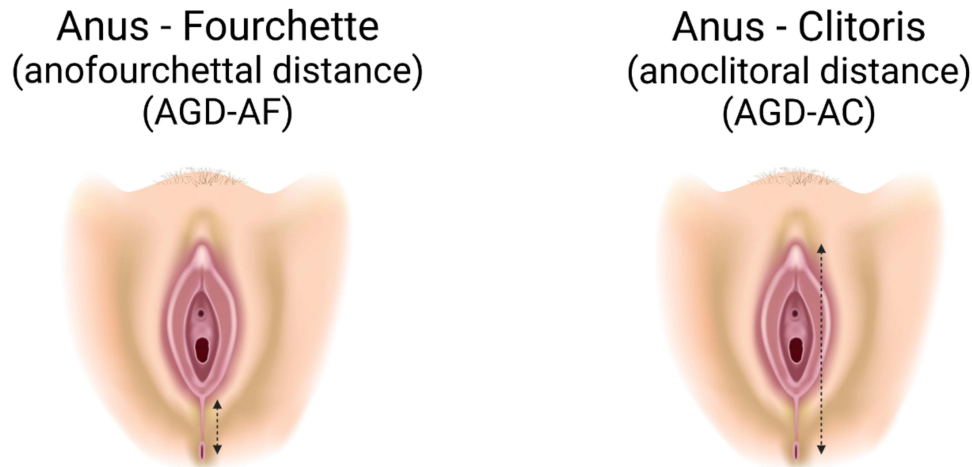


Figure 1 Depiction of measurements used for the two metrics of anogenital distance, in women.

Although the process of menstruation is mainly restricted to primates, the link between a low-androgen fetal environment, a short AGD, and reproductive effects is also supported by non-primate animal models. While in utero, the exposure of a female fetus to testosterone is affected by the presence or absence of adjacent male siblings and their higher levels of testosterone production. Female laboratory mice with no adjacent male fetuses, and thus with lower exposure to prenatal testosterone, exhibit shorter AGDs, lower postnatal serum testosterone, earlier vaginal opening, and shorter and more regular cycles, when compared with females flanked by one or two males in utero (reviewed in Crespi and Dinsdale²⁷). Similarly, female rats subject to lower testosterone in utero exhibit shorter AGDs, earlier vaginal opening, and shorter estrus cycles. In Mongolian gerbils, female fetuses flanked by either one or no males while in utero had lower postnatal adult serum testosterone, earlier onset of estrus and shorter cycles than female fetuses that were flanked by two males in utero.²⁷ These results parallel many of the patterns observed clinically among women with endometriosis, who, in addition to exhibiting shorter AGDs, also show evidence of earlier menarche, lower postnatal serum testosterone, and shorter, more regular menstrual cycles than controls.^{21,28–30} With regard to obstetric outcomes, Berlanda et al found a higher rate of preterm delivery in women with endometriosis that conceived naturally, when compared to women without endometriosis.³¹

In contrast to these results for endometriosis, women with polycystic ovary syndrome (PCOS), a disorder characterized by multifollicular ovaries, oligomenorrhea, and a relatively higher serum testosterone in adult life than controls, manifest multiple clinical features that are the opposite to those experienced by women with endometriosis.^{21,27,32} Women with PCOS exhibit longer AGDs, increased AMH, lower sex hormone binding globulin (SHBG), elevated luteinizing hormone (LH), increased waist to hip ratios (WHR), and increased body mass index (BMI) when compared to controls.^{21,32} Interpretation of these comparisons is complicated by the presence of both endometriosis and PCOS within some individuals.³³ However, as the prevalence of both conditions is multifactorial, additional factors may be present. For example, prolonged medical management of PCOS using hormonal therapies to induce more regular menstruation may increase exposure of the pelvic peritoneum to menstrual fluid and the enhanced development of endometriosis lesions. Systemic hormonal therapies may reduce androgen effect through the induction of SHBG in the liver and

increased androgen protein binding. In addition, the development of endometriosis lesions may be facilitated by the chronic low-grade inflammation present in PCOS women with increased BMI.

Reduced Androgen Levels as Outcomes of Genetic and Epigenetic Factors in Women with Endometriosis

Genetic factors mediate approximately 50% of the risk of developing endometriosis.³⁴ Genetic risk is mediated by the effects of many alleles each of small effect. Recent GWAS meta-analysis of 17,045 women with endometriosis and 191,596 controls has documented five novel genome-wide significant SNPs, that are enriched for a set of genes (including *FNI*, *CCDC170*, *ESR1*, *SYNE1* and *FSHB*) involved in steroid hormone levels and activities.³⁵ Of these genes, the *FSHB* gene is of particular interest, because the haplotype (genetic region) conferring higher endometriosis risk has also been significantly associated with lower serum testosterone, lower luteinizing hormone, heavier menstruations, shorter menstrual cycles, earlier menarche and earlier menopause, all of which characterize the endometriosis phenotype as noted above.^{30,36} This haplotype also confers a lower risk of PCOS.³⁷

More broadly, Mendelian Randomization analyses using endometriosis GWAS data can be used to help uncover the causal, genetic bases of the clinical correlates of endometriosis. These analyses have shown that endometriosis shares causal genetic risk factors with early menarche, shorter menstrual cycles, lower WHR, lower BMI, and lower levels of AMH.³⁸ As described above, lower AMH is independently associated with a shorter AGD among women (without endometriosis or PCOS) undergoing in vitro fertilization²²; AMH levels are also positively correlated with serum testosterone among reproductive-age women,^{39,40} and BMI is positively associated with serum testosterone among premenopausal women without reproductive disorders.^{41,42} Taken together with the data on AGD, these findings provide evidence that low testosterone and its correlates are involved in the genetic and developmental basis of endometriosis.

Low Androgens as a Consequence of Prenatal Endocrine Disruption

Endocrine disrupting substances, including organochlorines, with anti-androgenic or pro-estrogenic properties are ubiquitous in modern life and influence the risk of many disorders, including endometriosis.^{43,44} However, endometriosis has existed throughout history, prior to the development of these chemicals, and not all women with endometriosis have been exposed to high levels of organochlorines during their lifetime. Prenatal factors or factors present during the menstrual years of her mother may also not be present during the life of the woman affected by endometriosis. For example, prenatal exposure to the potent synthetic estrogen diethylstilbestrol (DES) notably increases endometriosis risk in offspring.⁴⁵

Prenatal exposure to the anti-androgenic chemical bisphenol A (BPA), results in shorter AGDs among female rats,⁴⁶ and, in mice, it has been linked with earlier first estrus and the production of endometriosis-like lesions.⁴⁷ In humans, prenatal BPA exposure in the first trimester leads to shorter AGD in daughters.⁴⁸

Prenatal exposure prenatally to phthalates, a set of mainly anti-androgenic agents, has been associated with shorter AGD in both mice and humans.⁴⁹ In contrast, a recent meta-analysis demonstrated only low-level associations between elevated phthalates and the presence of endometriosis in adult women,⁵⁰ although this research did not consider prenatal phthalate exposures. These findings support the relative importance of prenatal rather than postnatal exposure in the genesis of endometriosis lesions.

The Relationship Between Low Androgens, Pain and Pain-Related Symptoms in Women

Pain sensitivity is higher among women with endometriosis than in healthy controls.^{51,52} However, while endometriosis lesions are associated with pain and pain-related symptoms in the majority of affected women, there is no consistent relationship between the severity of endometriosis lesions and the severity of pain.⁵³ Additional factors across the biopsychosocial spectrum influence the pain experience, and multiple lines of evidence across both preclinical and human studies support increased chronic pain symptoms with reduced levels of testosterone.

For example, pain sensitivity is higher among women than men.^{51,52} Female sensitivity to pain varies with the menstrual cycle and correlates more closely with levels of the anti-nociceptive testosterone than levels of the pro-nociceptive estradiol.⁵⁴ Women with dysmenorrhea-related pelvic pain demonstrate strong inverse correlations between measures of chronic pain and androgen levels, especially for the correlation between days per month of pelvic pain and the free androgen index which measures the unbound fraction of testosterone within blood.^{55,56}

Experimental and observational studies in humans and animal models have consistently shown that treatment with testosterone can ameliorate chronic pain in a proportion of individuals. Female-to-male transsexuals report lower levels of chronic pain following hormonal transition using testosterone therapy, while male-to-female transsexuals report increased pain.⁵⁷ Testosterone therapy has been used to reduce pain in opioid-induced chronic pain⁵⁸ and fibromyalgia,⁵⁹ both centrally mediated pain conditions. Preclinically, an experimental study of female rats exposed prenatally to testosterone showed reduced pain responses in adult life that were similar to those of males.⁶⁰ This latter study is especially important because it establishes the key importance of prenatal, organizational effects of testosterone on adult pain sensitivity, and leads to the prediction that shorter anogenital distance in humans, as a metric of lower prenatal testosterone, should be associated with higher sensitivity to pain.

A potential mechanism for the pain-modulating effect of androgens on chronic pain is via their inverse association with levels of systemic immune-based inflammation.⁶¹ Androgens are considered to be generally immunosuppressive, resulting in decreased T- and B-cell proliferation, and decreased immunoglobulin and cytokine production.⁶² A higher prevalence of autoimmune disorders is found in females when compared to males,⁶³ in males or females with low androgen levels^{64–66} and in women with endometriosis.^{67–69} Testosterone has been shown to reduce inflammation, as measured by levels of the pro-inflammatory cytokine IL-1 β and pain symptoms in patients with rheumatoid arthritis.

An additional mechanism for the effects of testosterone on pain may be through their links with β -endorphin levels within the central nervous system. Pluchino et al⁷⁰ showed that testosterone administration increased β -endorphin levels in the brains and plasma of ovariectomized rats. In human studies, pain sensitivity in women with endometriosis has been associated with lower levels of β -endorphins,⁵³ whereas PCOS is associated with higher levels of β -endorphins.^{71–74} Although some studies have been inconclusive,^{75,76} the opioid antagonist naltrexone is effective in reducing endocrine symptoms in women with PCOS,^{77–79} also suggesting that high β -endorphin levels are a feature of this condition. Taken together, the studies described above provide diverse evidence suggesting that the pain symptoms of endometriosis may derive, at least in part, from low testosterone, through both prenatal and postnatal effects.

Androgens and Fertility in Women with Endometriosis

Optimal levels of serum and ovarian androgens, especially testosterone, play key roles in additional aspects of female reproductive development, functions and disease.^{8,9} Testosterone is essential for folliculogenesis,⁸⁰ and low levels of testosterone are associated with both apoptosis of follicular granulosa cells,⁸¹ and reduced implantation success of embryos.⁸²

A shorter AGD, representing low androgen levels during fetal development, is directly related to a set of fertility correlates including higher risk of premature ovarian insufficiency,^{23,83} and poor ovarian response to controlled ovarian stimulation,²² consistent with roles in folliculogenesis. Moreover, by a recent meta-analysis, testosterone therapy increased IVF success in women with a poor response to ovarian stimulation.⁸⁴ These convergent results suggest that low prenatal testosterone, with sequelae that include altered programming of the hypothalamic-pituitary-ovarian axis during fetal development, and consequent reduced post-natal testosterone levels and higher levels of pain, results in a broad set of sexually dimorphic traits that may partially underlie the reduced fertility found in women with endometriosis (Figure 2).

Currently, the best-known correlates of low amniotic fluid testosterone among female fetuses are young maternal age, low weight gain during pregnancy, and low amniotic fluid cortisol; these factors together account for 64% of variation in testosterone level.⁸⁵ However, the relationship between amniotic fluid testosterone and fetal AGD remains unknown.

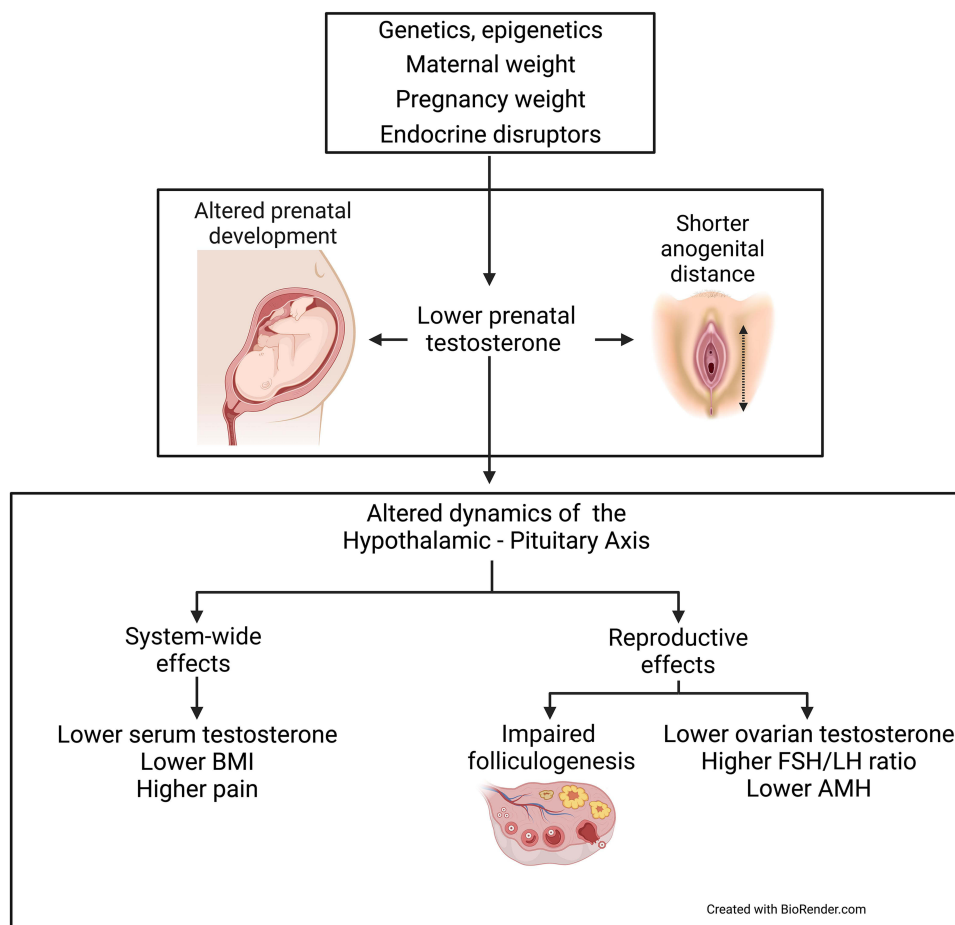


Figure 2 Lower prenatal testosterone is caused by a variety of factors, and mediates a suite of effects on the development of female physical and reproductive traits, including increased risk for endometriosis.

Translational Implications for Research and Treatment

Probably the most important long-term implication of the recent findings that link endometriosis with low prenatal testosterone is that they begin to establish endometriosis as a developmental condition, within the general paradigm of DoHAD, the developmental origins of health and adult disease.⁸⁶ A DoHAD framework for studying and treating endometriosis compels a focus on early-developmental programming of the HPO axis by testosterone, in the context of KNDy neuron activity and pulsatile secretion patterns of GnRH (eg, Cernea et al⁸⁷). This paradigm is relatively well developed with regard to PCOS, especially by the development of animal models that recapitulate major features of the disease in humans through experimental increases in prenatal testosterone.⁸⁸ In principle, comparable animal models can be developed for the analysis of endometriosis, built around the downstream effects of reduced testosterone during early prenatal development. Importantly, the DoHAD paradigm has yet to address prenatal programming of pain sensitivity, despite its importance in disease and well-being.

Risal et al⁸⁹ and Parker et al⁹⁰ recently published on the transgenerational inheritance of PCOS, a condition with genetic pre-disposition but potential for amplification by the developmental environment: in utero exposure to elevated testosterone levels. The risk of endometriosis, a condition also showing a strong genetic predisposition, appears, in contrast, to be augmented by exposure to relatively low testosterone levels in utero. These findings provide the potential for alleviation of endometriosis among high-risk individuals, through modulation of factors that mediate levels of testosterone and other androgens during prenatal as well as postnatal development.

The potential to influence a woman's androgen levels offers the possibility of reduced pain symptoms, improved fertility and reduced lesions. In 1971, the synthetic androgen danazol was the first drug approved for treating

endometriosis.⁹¹ This treatment was effective in reducing pain among about 90% of the women with endometriosis.⁹² However, as a proportion of women experienced androgenic adverse effects, and with the advent of GnRH analogue drugs, the focus of treatment moved from administration of androgens to suppression of estrogen. The recent resurgence of interest in roles for testosterone as treatment for chronic pain and infertility, with its relatively advantageous hormonal profile when compared with danazol, suggests that a new, more nuanced role for androgen therapy in a proportion of women with endometriosis or associated pain-related symptoms may be possible.^{55,56} Navigating a path forward with menstrual suppression, reduced estrogen effects, enhanced androgen effects, and minimization of intralesional conversion of testosterone to estradiol via the enzyme aromatase will require innovative developments in hormonal therapeutics.

Conclusions and Future Questions

The links described here between a shorter anogenital distance and an individual woman's risk of endometriosis immediately raise a number of key questions. Do women with endometriosis with shorter AGDs consistently show lower serum or ovarian testosterone? Do they experience higher levels of pain? Is lower testosterone in mothers a risk factor for endometriosis and a shorter AGD in their daughters, in the same general way that longer AGD, and higher prenatal testosterone, have been shown to mediate transgenerational effects on risk for PCOS?⁸⁹ Could modification of lifestyle-related causes of variation in prenatal testosterone levels reduce risk of developing endometriosis lesions during adult life? And might high levels of pain in many autoimmune conditions,⁹³ which are strongly female biased in prevalence and have been linked with low adult testosterone (eg, Tomassini et al⁹⁴), also be linked with prenatal testosterone?

Additional studies using rodent models of endometriosis might usefully analyze the endocrine correlates of an especially low AGD in more detail. Do short-AGD females of such species show increased pain and inflammation, and more-pronounced development of ectopic endometrial implants than those with higher AGD? Do they show alterations to KNDy neurons, and GnRH-system regulation, that could provide new insights into the developmental predispositions and neuroendocrine origins of this disorder?⁹⁵ The new perspectives described here are highly predictive and should help guide both laboratory research and clinical studies in developing better ways to alleviate endometriosis.

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Author Contributions

All authors made significant contributions to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Dr Susan Evans reports that she is a minor shareholder of Havah therapeutics which is commercialising a testosterone product for use in breast cancer treatment. The authors report no conflicts of interest in this work.

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