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INVITED REVIEW

Male Fertility

The role of estradiol in male reproductive function

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Traditionally, testosterone and estrogen have been considered to be male and female sex hormones, respectively. However, estradiol, the predominant form of estrogen, also plays a critical role in male sexual function. Estradiol in men is essential for modulating libido, erectile function, and spermatogenesis. Estrogen receptors, as well as aromatase, the enzyme that converts testosterone to estrogen, are abundant in brain, penis, and testis, organs important for sexual function. In the brain, estradiol synthesis is increased in areas related to sexual arousal. In addition, in the penis, estrogen receptors are found throughout the corpus cavernosum with high concentration around neurovascular bundles. Low testosterone and elevated estrogen increase the incidence of erectile dysfunction independently of one another. In the testes, spermatogenesis is modulated at every level by estrogen, starting with the hypothalamus-pituitary-gonadal axis, followed by the Leydig, Sertoli, and germ cells, and finishing with the ductal epithelium, epididymis, and mature sperm. Regulation of testicular cells by estradiol shows both an inhibitory and a stimulatory influence, indicating an intricate symphony of dose-dependent and temporally sensitive modulation. Our goal in this review is to elucidate the overall contribution of estradiol to male sexual function by looking at the hormone's effects on erectile function, spermatogenesis, and libido.

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ASSOCIATION BETWEEN LIBIDO AND ESTRADIOL

Role of estradiol in the brain

The effect of estradiol on libido is seen at various levels of regulation, starting with direct effects in the brain (Figure 1). Areas of the brain that control sexual behavior in mammals are thought to do so via pheromones that induce specific sexual effects on the autonomic nervous system, including changes in mood and sexual arousal. Pheromones produce increased activity in the medial preoptic area/anterior hypothalamus.¹ Neurons, the most basic electrical information-transmitting cells in the central nervous system and peripheral nervous system, as well as astrocytes, star-shaped glial cells which fulfill a number of functions in the central nervous system, both convert testosterone to estrogen with aromatase. The preoptic area and anterior hypothalamus contain the highest levels of aromatase and estrogen receptors (ERs) in male rodents.^{2,3} Similarly, it is well known that selective serotonin reuptake inhibitors diminish libido. Serotonin receptors follow a pattern of distribution similar to that of ERs in the brain.⁴ However, the interaction of estradiol and serotonin is complex and will subsequently be addressed. Finally, aromatase activity is highest in the brain during development. Thus, not only does estradiol modulate sexual behavior in the adult male, it also appears to organize the early brain to program sexual behavior.³

Estradiol effect at low testosterone levels

To discern the effect of estradiol, it is important to evaluate its effect on libido at both low and normal levels of circulating testosterone. Decreased testosterone is clearly associated with low libido in males.⁵ In men with diminished testosterone, the administration of exogenous

estradiol has been shown to increase libido.⁶ This finding is supported by rodent studies demonstrating that castrated animals given exogenous estrogen show an increase in sexual activity in a dose- and temporal-dependent manner.⁷ In addition, in a unique case report of a male patient with aromatase deficiency and hypogonadism, both estrogen and testosterone were required to increase libido, whereas neither hormone could achieve the effect alone suggesting that estrogen plays a necessary role in sexual desire in the setting of low testosterone.⁸

Similarly, patients with prostate cancer treated with androgen deprivation therapy (ADT) serve as a good model for the influence of estrogen on libido. When castrate levels of androgens were reached ($T < 50 \text{ ng dl}^{-1}$), uniform adverse effects of hot flashes, erectile dysfunction (ED), and decrease in libido were reported.⁹ When comparing androgen receptor (AR) blockers versus castration, the former had better outcomes in maintaining sexual activity, presumably by increased testosterone conversion to estrogen.¹⁰ This evidence, though indirect, does perhaps suggest that elevated estrogen in men with low or absent testosterone can sustain libido. In addition, administering estradiol to men undergoing ADT for prostate cancer could possibly reduce damage to areas of the brain associated with sexual performance. Thus, an overall increase in sexual quality of life could be achieved.⁶

Role of estradiol in eugonadal men

While estradiol has been shown to have a positive effect on libido at low levels of testosterone, a limited number of studies have looked into the effect of estradiol supplementation in eugonadal men and reported conflicting results. One study with continuous estradiol administration

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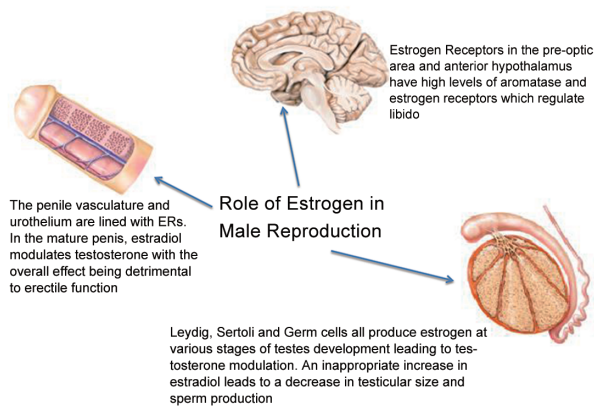


Figure by Vanessa Dudley

Figure 1: The role of estrogen in male reproduction.

in men who had normal testosterone levels showed decreases in sexual interest, fantasy, masturbation, and erections.¹¹ In contrast, a randomized, double-blind study conducted on 50 men ages between 20 and 40 years demonstrated that sexual activity was unaffected.¹²

Uncontrolled case reports also have shown conflicting results. A man with aromatase deficiency was noted to have a relevant increase in sexual behavior with estrogen supplementation,¹³ while other aromatase-deficient men noted no change in their sexual function.¹⁴ These natural models, which have the potential to provide some clarity, along with results of the limited trials undertaken, have not provided definitive evidence one-way or the other regarding estradiol's effects on libido in the eugonadal male.

Role of estradiol in hypogonadal men treated with testosterone supplementation therapy

Perhaps, most relevant to the discussion is the use of testosterone supplementation therapy (TST). The goal of TST, regardless of the method used, should be to maintain not only physiologic levels of testosterone, but also its metabolites, including estradiol which optimizes libido.¹⁵

In men with secondary hypogonadism (functioning testes and relatively low levels of luteinizing hormone [LH] and testosterone), clomiphene citrate was used to increase testosterone by acting centrally on the ER weakly. Clomiphene citrate administration raised endogenous testosterone while increasing the testosterone to estradiol (T/E) ratio.¹⁶ Also, in a later study, clomiphene citrate administered to hypogonadal men produced an increase in libido, energy, and sense of well-being.¹⁷

In 2013, Finkelstein *et al.* looked at the effects of testosterone and estrogen on male sexual function. They found that the administration of testosterone with and without aromatase inhibitors markedly impaired sexual function when aromatization was inhibited.¹⁸ In addition, a study by Ramasamy *et al.* in 2014 showed that libido was increased in men receiving TST when testosterone levels were >300 ng dl⁻¹ and estradiol levels were >5 ng dl⁻¹. Most compelling is the fact that in men with serum testosterone <300 ng dl⁻¹, sexual drive was seen to be markedly higher when estradiol levels were >5 ng dl⁻¹.¹⁹ In addition, when patients with low testosterone were treated with letrozole, a potent aromatase inhibitor, libido was decreased, suggesting that complete elimination of estradiol and decreasing the T/E ratio too severely, adversely affects sexual desire in men.²⁰ These studies provide evidence that both estrogen and testosterone are necessary for normal libido in testosterone-deficient men. Clinically, the dependence of libido in

hypogonadal men on both testosterone and estrogen indicates that a cautious approach to the use of aromatase inhibitors is warranted and that the T/E ratio has an impact. It might be reasonable that while prescribing TST one should monitor the levels of both testosterone and estrogen and their relationship to each other.

Clearly, the effect of estradiol on male sexual desire is linked to testosterone levels, as there are different outcomes when estrogen is administered at low and normal testosterone levels. Another example of this duality is seen in men with androgen resistance, where unfettered estrogen is able to stimulate subsequent breast development. However, in men with normal androgen receptor activity, estradiol is unable to stimulate breast development.²¹ This is thought to be due to an imbalance between the inhibitory and stimulatory effect of these hormones.^{22,23} Whatever the pathophysiology in breast development or libido, these hormones seem to be inextricably linked in the complicated physiology of male sexuality and development.

Finally, the effect of estradiol on mood must be considered. As mood can correlate with sexual interest, it is reasonable to consider these data when discussing the role of estradiol on libido. While cognition, well-being, and depressive symptoms improve in men whose low testosterone levels were corrected,²⁴⁻²⁶ higher levels of estrogen also have been associated with less depression in older patients of both sexes.²⁷ In addition, estrogen supports serotonin levels and affects the amount of 5-HT receptors in the brain, and depending on receptor subtype, there is sexual inhibition or facilitation.²⁸⁻³⁰ A recent study showed a significant positive correlation between endogenous plasma estradiol levels and cortical 5-HT_{2A} binding in men, with no independent effects on these receptors from testosterone.³¹ In addition, when serotonin binds to these 5-HT_{2A} receptors in the cortex, limbic system, hypothalamus, and midbrain, sexual desire is inhibited with subsequent induction of refractoriness and sexual satiety.³² The interaction of estrogen with serotonin is complex, with overlapping influences that reaches beyond sexual desire including mood regulation and cognition.³³ This fact makes its true impact on sexual desire and behavior difficult to fully elucidate.

ASSOCIATION BETWEEN ESTRADIOL AND ERECTILE FUNCTION

Erectile function is multifaceted with a necessary combination of nerve, vessel, and endocrine actions that work together to produce subsequent penile structural changes in a coordinated fashion. Smooth muscle, endothelium, and cell-to-cell communications via gap junctions are essential to erectile function, and thus pathology in any one of these can lead to ED.³⁴ The pathophysiology and clinical role of testosterone in erectile function have been studied extensively.³⁵⁻³⁷ Androgens are necessary for the penis to grow and develop, and also contribute to the physiology of erections.³⁸

Estrogen in animal models impedes normal penile development, including reduced bulk of the bulbospongiosus muscle, reduction of the spaces in corpus cavernosum, and an accumulation of fat cells within existing spaces that lead to ED in adult life. Notably, the reported exposure was limited to early development, and rats that were exposed to exogenous estradiol after day 12 of life showed no structural abnormalities.³⁹ In addition to influence on structure, estrogen has a significant influence on penile vasculature. A case-control study of male outpatients with ED with venous leakage showed that the only difference between the men with and without ED was an increased estradiol level. The authors concluded that estradiol increases venous vascular permeability via VEGF and has a detrimental effect on erectile function through increased venous leakage.⁴⁰

Estrogen also acts at the level of the brain to influence erectile function. Estrogen inhibits the hypothalamus-pituitary axis and subsequently follicle-stimulating hormone (FSH) and LH, thus reducing circulating testosterone.^{41–43} Testosterone is necessary for normal erectile function, and low testosterone produces decreased firmness, ability to maintain erections and number of erections achieved, all of which are improved with testosterone administration.^{44,45} The administration of exogenous estradiol has the ability to cause ED through an inhibitory effect on testosterone production. This was demonstrated in animals, where exogenous estrogen administration not only reduced testosterone levels, but also diminished the structural integrity of the corpus cavernosum with less viable smooth muscle and an increase in connective tissue.⁴⁶ Similarly, in humans taking estrogen, a reduction is seen in spontaneous erections and nocturnal penile tumescence correlating with a reduction in testosterone levels.⁴⁷

The effects of estrogen on erectile function discussed above largely occur as a function of its ability to decrease circulating testosterone. In addition, increased severity of ED, assessed with the International Index for Erectile Function,⁴⁸ in men with low testosterone levels is worsened with high estrogen levels implying an additive effect by the two hormones.⁴⁹ However, while low testosterone increases the incidence of ED, elevated estrogen levels do as well.⁴⁰ This point, coupled with the fact that the corpus cavernosum vasculature and urothelium have extensive ERs, significantly more than other steroid receptors, and particularly around the neurovascular bundle, suggests mechanisms of ED separate from and in addition to central testosterone inhibition.^{50,51}

Evidence from animal models demonstrates this independent role of estradiol in erectile function. Stimulation of ER receptors has been shown to have anti-apoptotic effects on endothelium, and the loss of this function in the crura was associated with ED.⁵² Similarly, when ED was induced by high estrogen levels, testosterone therapy was not helpful in restoring erections as long as the estrogen milieu was maintained.⁵³ While animal models have not provided conclusive evidence, and the pathophysiology of erectile function is more complex than simple hormone difference, these findings provide an interesting and compelling initial step in elucidating estrogen's separate and direct effect on ED outside of its role in testosterone suppression (**Figure 1**).

ROLE OF ESTRADIOL IN SPERMATOGENESIS

Testosterone has long been known to be the dominant sex hormone in men. However, estrogen is found at detectable levels at multiple points of development and contributes to spermatogenesis (**Figure 1**). In addition, an abnormal T/E ratio (<10) has been associated with decreased semen parameters, and administration of an aromatase inhibitor normalized the ratio and improved sperm concentration, motility, and morphology.²⁰ Targeting estrogen levels has clinical value when optimizing sperm retrieval rates in men with nonobstructive azoospermia (NOA). Sperm retrieval rates were seen to increase 1.4-fold either by decreasing estradiol directly and normalizing the T/E ratios with aromatase inhibitors or indirectly blocking estradiol centrally with clomiphene citrate, in turn, increasing gonadotropin secretion.⁵⁴ Although it has not been made clear if the improvement in spermatogenesis is specifically due to the reduction of estradiol in the testes when normalizing the T/E ratio, it is clear that estrogen levels play an important and modifiable clinical impact on spermatogenesis in men with NOA. In addition, varicocele has long been known to have an adverse impact on fertility and sex hormone production, and while the mechanism of this affect is unknown, estrogen has been linked.^{55,56} Semen analysis of “varicocele” sperm showed a marked reduction in both ER alpha and beta receptors as well as

a reduced response to estradiol's necessary effects on motility and acrosin activity.⁵⁶

Estradiol has been shown to exist not only in the reproductive tract of the adult male, but in the brain as well.⁵⁷ In various species including humans, a more significant concentration exists in the male reproductive tract and semen than in the serum.⁵⁸ While it has been shown clearly that Leydig and Sertoli cells produce estradiol, newer research has demonstrated that estradiol synthesis by germ cells within the seminiferous tubules contributes significantly to the hormonal milieu within the tubules (**Figure 2**).⁵⁹ Aromatase has also been found in early development in all germ cells, especially during meiotic and postmeiotic stages of spermatogenesis, and in the later development of ejaculated spermatozoa.⁶⁰ However, it is the presence of ERs in these cells and their precursors that provides compelling evidence that estrogen has an influence over spermatogenesis.⁶¹

Leydig cells – self-regulation and testosterone control via estradiol

Leydig cells, under the influence of LH, secrete testosterone, which in turn acts on Sertoli and peritubular cells, as well as vasculature, allowing them to nurture the budding spermatogonia to spermatozoa.⁶² At birth, fetal Leydig stem cells are not progenitors of the adult form. However, they remain in small numbers in the adult testes as a distinct cell population that is important for the generation of their adult counterparts.⁶³ Aromatase is present largely in mature Leydig cells, producing a significant amount of the estradiol in the testes.^{64,65} In addition, ER mRNA has been shown to exist in both cell precursor and mature populations.⁶⁶ When subjected to an alkylating agent toxic to Leydig cell populations, male rats which then had subsequent estradiol exposure during a critical stage of development experienced a blockage in the reappearance of mature Leydig cells.⁶⁷ Thus, Leydig cells, at least in part, self-regulate via estrogen modulation by potentially controlling the extant population of fetal Leydig cells in a paracrine fashion. Furthermore, there is evidence suggesting that estrogen inhibits the LH effect on Leydig cells⁶⁸ and that excess estrogen exposure reduces serum testosterone levels via this inhibition. The subsequent reduction of testosterone in turn reduces the number of viable sperm.^{69,70} Taken together, the evidence shows that estrogen plays a major role in the overall growth, development, and function of Leydig cells, in one role acting as a modulator of precursor populations and, in another role inhibiting steroidogenesis via the effect of LH on the mature Leydig cells, ultimately producing a net effect of androgen inhibition.⁷¹

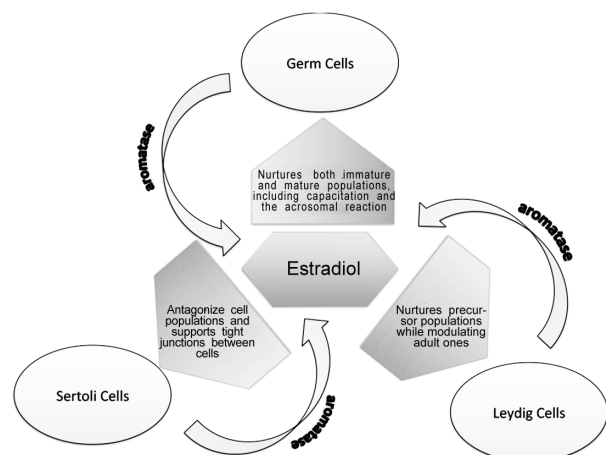


Figure 2: The role of estradiol in spermatogenesis.

Sertoli cells – contributions of estradiol to the immature testes and sperm maturation

While a major source of estrogen in the adult testes is the Leydig cell, Sertoli cells produce most of the estrogen in the immature testes.⁶⁰ In multiple species, aromatase mRNA was detected in Sertoli cells at every stage of sperm development.⁷² In addition, it has recently been shown that ERs, specifically the beta type and G protein estrogen receptors are present in male rats' Sertoli cells during development and are subjected to modulation by estradiol in concert with FSH.^{73–75} The overall effect of this modulation is inhibition, as shown by the antagonism of estrogen in neonatal animal testes with subsequent increase in Sertoli cell population.^{76,77} In addition, male neonatal rat testes exposed to estradiol displayed a subsequent dose-dependent reduction of 20%–70% of testes weight. A reduction of sperm production with poor motility was also dose-dependent, with the upper range doses of estradiol producing complete azoospermia. These changes are androgen-dependent and have been linked to a down regulation of ARs on Sertoli cells via the effect of estradiol on the coexisting ERs.⁷⁸

Finally, it is known that Sertoli cells must “recognize” developing sperm, as the perpetual and cyclical topological relationship is directly related to the maturation process of spermatogonia.⁷⁹ This process is essential as the immature sperm progress in the apical direction, mature, and eventually secrete into the tubules. It has been shown that FSH in combination with estradiol is necessary for the mRNA transcription of N-cadherin, the protein responsible for cell-to-cell adhesion.^{80,81} Thus, the dynamic process of spermatogenesis involving Sertoli cells separating and reforming tight junctions via N-cadherins is, at least in part, regulated by estrogen.

Germ cells – autocrine and paracrine effects of estradiol

Testosterone produced by Leydig cells and FSH from the anterior pituitary are necessary for Sertoli cells to transduce signals and produce factors that nurture germ cells.^{82,83} In addition, when testosterone is withdrawn from germ cells by administering ethane 1,2-dimethanesulfonate, an alkylating agent that selectively kills Leydig cells in adult rat testes, apoptosis is induced via the Fas/Bcl-2 system.⁸⁴ While this requirement of testosterone for germ cell survival is well established, evidence of estrogen's effect on germ cells is a more recent discovery. Aromatase has been confirmed in the cytoplasm surrounding elongated spermatids, as well as ejaculated sperm, and a link exists between estradiol production in sperm and capacitation and the acrosome reaction. When placed in a noncapacitating medium, only estradiol and aromatizable steroids were able to increase sperm motility and migration, making estradiol necessary for sperm maturation and successful fertilization.⁸⁵

In the neonatal period, the major portion of estradiol is synthesized by Sertoli cells, and germ cell precursors are stimulated through plasma membrane ERs.⁸⁶ In addition, as germ cells multiply and begin producing estradiol, the hormone inhibits aromatase in Sertoli cells in a paracrine fashion to allow them subsequently to proliferate and nurture the spermatogonia to maturity.⁸⁷ Along with estradiol from Leydig cells, the estrogens produced by germ cells allow for autocrine self-stimulation, thus creating a positive feedback loop promoting germ cell and subsequent paracrine Sertoli cell propagation.⁸⁸

Unlike estrogen's inhibitory influences on Leydig and Sertoli cells, as described above, a stimulatory effect is seen with germ cells. Like Sertoli and Leydig cells, ERs and aromatase are found in germ cells, again suggesting the production of as well as modulation by estradiol.^{86,89,90} It was once thought that Leydig cells produced most of the estrogen in the adult testes, but new evidence shows that germ cells

produce anywhere from 50% to 60% of the hormone.^{60,91} Most recently, it has been demonstrated that estradiol, along with platelet-derived growth factor (PDGF), stimulates germ cell proliferation that is dose-dependent and nonadditive, giving Sertoli cells a way to regulate the germ cells' entry into mitosis via estradiol production.^{92,93} When incubating mature human seminiferous tubules without survival factors, even low concentrations of estradiol effectively enhanced germ cell production by the inhibition of apoptosis. Thus, estrogen has proven to be a potent hormone necessary for germ cell survival.⁹⁴ Seemingly, contradictory to this is the fact that excess estrogen acts as an inhibitor of spermatogenesis in human⁹⁵ as well as rat models.^{70,78,96} However, it is likely the inhibitory effect of estrogen on Sertoli cells, rather than on germ cells, that causes the overall inhibition of spermatogenesis.

An interesting case report from the *New England Journal of Medicine* describing a man with an estrogen receptor mutation with normal sexual development and relatively normal semen parameters warrants discussion. While the patient had a low normal sperm density, a seemingly contradictory fact to the evidence presented above, his sperm viability was well below normal at 18%, and fertility could not be assessed.⁹⁷ In male mouse models, the targeted disruption of estrogen receptors led to alteration in spermatogenesis and infertility; however, in human models, many different novel mutations in ERs have been identified, some in infertile men and some in men with normal fertility.^{98,99} Perhaps, this means relatively normal sexual parameters can be explained by a mutation or receptor type that does not confer infertility. The authors did not address that, however this point once again addresses the complexity of estradiol on male sexual function and demonstrates why further study is needed.

CONCLUSIONS

The exact role of estradiol in each area of male sexual function including libido, erectile function, and spermatogenesis, is difficult to determine. A complex balance of testosterone, estradiol, aromatase, and ERs in the testes, penis, and brain confirms an indispensable and highly regulated hormonal interaction of estrogen in the male. ERs and aromatase share topographic locations with pheromones in the brain, making it clear that estrogen contributes to early sexual development as well as sexual behavior in adulthood. Estrogen can sustain libido as well as affect the amount of serotonin receptors in the brain modulating mood, mental state, cognition, and emotion. Erectile function is adversely affected by estrogen exposure in early penile development, and exposure to estradiol in the mature penis leads to increased vascular permeability with increased ED. ED from increased estradiol exposure is independent of testosterone level. In addition, spermatogenesis is dependent upon estradiol to some extent, as all cells involved in the process of sperm production contain aromatase and express ERs. Finally, estradiol levels should be considered when treating men with TST, as estradiol levels below 5 ng dl⁻¹ correlate to a decrease in libido. Considering the complexity and taking into account some conflicting data, more research is necessary so that when better understood, estradiol can become clinically useful in treating diminished libido, ED, and perhaps even oligospermia.

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