


Clinical application of aromatase inhibitors to treat male infertility

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BACKGROUND: Infertility affects 15% of men and contributes to nearly half of all cases of infertility. Infertile men usually have impaired spermatogenesis, presenting as azoospermia or various degrees of asthenospermia and oligozoospermia. Spermatogenesis is a complex and coordinated process, which is under precise modulation by the hypothalamic–pituitary–gonadal (HPG) axis. An aberrant hormone profile, especially an imbalance between testosterone (T) and estradiol (E₂), plays an essential role in male infertility. In the male, E₂ is produced mainly from the conversion of T by the aromatase enzyme. Theoretically, reducing an abnormally elevated T:E₂ ratio using aromatase inhibitors (AIs) could restore the balance between T and E₂ and optimize the HPG axis to support spermatogenesis. For decades, AIs have been used to treat male infertility empirically. However, owing to the lack of large-scale randomized controlled studies and basic research, the treatment efficacy and safety of AIs in male infertility remain controversial. Therefore, there is a need to summarize the clinical trials and relevant basic research on the application of AIs in the treatment of male infertility.

OBJECTIVE AND RATIONALE: In this narrative review, we summarized the application of AIs in the treatment of male infertility, including the pharmacological mechanisms involved, clinical trials focused on patients with different types of infertility, factors affecting treatment efficacy and the side-effects.

SEARCH METHODS: A literature search was performed using MEDLINE/PubMed and EMBASE, focusing on publications in the past four decades concerning the use of AIs for treating male infertility. The search terms included AI, male infertility, letrozole, anastrozole, testolactone, azoospermia, oligozoospermia, aromatase polymorphisms, obesity and antiestrogens, in various combinations.

OUTCOMES: Clinical studies demonstrate that AIs, especially nonsteroidal letrozole and anastrozole, could significantly inhibit the production of E₂ and its negative feedback on the HPG axis, resulting in increased T and FSH production as well as improved semen

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parameters in infertile men. Large-scale surveys suggest that obesity may result in symptoms of hypogonadism in both fertile and infertile males, such as decreased semen quality and attenuated sexual function, which can be improved by AIs treatment. Polymorphisms of the aromatase gene *CYP19A1*, including single nucleotide polymorphisms and tetranucleotide TTTA repeats polymorphism (TTTAn), also influence hormone profiles, semen quality and treatment efficacy of AIs in male hypogonadotropic hypogonadism and infertility. The side-effects of AIs in treating male infertility are various, but most are mild and well tolerated.

WIDER IMPLICATIONS: The application of AIs in treating male infertility has been off-label and empirical for decades. This narrative review has summarized the target patients, dose, treatment duration and side-effects of AIs. Polymorphisms of *CYP19A1* that may affect AIs treatment efficacy were also summarized, but a full understanding of the mechanisms involved in AIs action requires further study.

Key words: male infertility / aromatase inhibitors / hypothalamic–pituitary–gonadal axis / spermatogenesis / hypogonadism / obesity / *CYP19A1* polymorphisms

Introduction

Infertility affects 15% of men and contributes to nearly half of all cases of infertility (Barratt et al., 2017). Various factors and clinical entities contribute to male infertility, including but not limited to varicocele, inflammation, genetic disorders such as Klinefelter syndrome and Y chromosome microdeletion, and congenital abnormalities, e.g. cryptorchidism, as well as environmental factors such as radiation and hyperthermia (Docampo and Hadziselimovic 2015; Jensen et al., 2017; Agarwal et al., 2018; Choy and Eisenberg, 2018; Krausz and Riera-Escamilla, 2018; De Felice et al., 2019). These pathogenic factors usually lead to impaired spermatogenesis, presenting as azoospermia or varying degrees of oligozoospermia, teratozoospermia and asthenozoospermia. Among them, non-obstructive azoospermia (NOA) is the severest type of impairment (Esteves, 2015). Notably, 43–45% of patients with oligozoospermia and NOA have been found to be hypogonadal, presenting as impaired testicular function and testosterone (T) synthesis (Nieschlag and Nieschlag, 2010). Although IVF and microsurgical testicular sperm retrieval (micro-TESE) combined with ICSI have enabled fertility for many couples, these techniques are still expensive and come with their own challenges and limitations (Flannigan et al., 2017; Niederberger et al., 2018; Schlegel et al., 2021). Also, many patients with NOA or severe oligozoospermia would barely benefit from these techniques. As such, medical therapies that improve spermatogenesis to sustain natural conception or decrease the level of ART necessary to achieve a pregnancy are required, and these are still limited (Tournaye, 2012; Pan et al., 2018).

It is known that spermatogenesis is a complex, coordinated process leading to the continuous production of spermatozoa, and it depends on an intact and well balanced hypothalamic–pituitary–gonadal (HPG) axis (Jarow and Zirkin, 2005; Neto et al., 2016; Wang et al., 2018). GnRH is secreted from the hypothalamus to stimulate the pituitary to produce LH and FSH (Yen, 1975; Skorupskaitė et al., 2014). In men, LH stimulates Leydig cells in the testis to produce testosterone (T), which is necessary for spermatogenesis (Mendis-Handagama, 1997; Ramaswamy and Weinbauer, 2014). A fraction of T is converted to estradiol (E_2) under the catalytic action of the enzyme aromatase (Carreau et al., 2003). FSH is crucial to maintain normal functions of Sertoli cells, a core component of the testis microenvironment or niche that supports spermatogenesis (Griswold, 1998; Walker and Cheng, 2005).

Testosterone and E_2 generate negative feedback to the pituitary and hypothalamus, resulting in decreased production of FSH and LH (Allan et al., 2010). Additional HPG feedback mechanisms exist for inhibin

and activin, two molecules produced by Sertoli cell to negatively and positively regulate the HPG axis, respectively (Fig. 1) (Toulis et al., 2010; Hedger and Winnall, 2012). Strikingly, E_2 has been demonstrated to provide a more powerful negative feedback on the HPG axis than T, reflecting its indispensable role in regulating spermatogenesis (Raven et al., 2006). However, increased E_2 levels resulting from excess aromatase activity have been observed in a proportion of infertile men, especially patients with NOA or oligozoospermia, presenting as reduced T: E_2 ratio (Pavlovich et al., 2001). Reducing abnormally elevated E_2 level via inhibiting aromatase conversion of T to E_2 or blocking E_2 effects on central receptors could repress the excessive negative feedback on the HPG axis, leading to improvement of spermatogenesis and relief of hypogonadism symptoms. Theoretically, this can be attained by administration of aromatase inhibitors (AIs) or selective estrogen receptor modulators (SERMs), which have been used to treat male infertility or hypogonadism empirically for decades (Schlegel, 2012; Cannarella et al., 2019; Schlegel et al., 2021). In this narrative review, we mainly discuss the application of AIs in the treatment of male infertility and hypogonadism, including the pharmacological mechanisms, clinical trials aimed at patients with different types of infertility, factors affecting treatment efficacy and side-effects.

Methods

A PubMed/MEDLINE and EMBASE search was performed using the keywords aromatase inhibitor, male infertility, letrozole, anastrozole, testolactone, azoospermia, oligozoospermia, aromatase polymorphisms, obesity, hypogonadism and antiestrogens in various combinations. Full articles, including clinical trials (randomized or non-randomized), case reports and case series, were retrieved and reviewed. In addition, registered clinical trials (from database *clinicaltrials.gov*) exploring the treatment efficacy of AIs in male infertility and hypogonadism were also reviewed.

Roles of aromatase and estrogen in the male reproductive system

The expression and function of aromatase

Aromatase is the sole member of family 19 of the P450 superfamily of enzymes, termed *CYP19A1* (Bulun et al., 2004). In humans, aromatase is present in the endoplasmic reticulum of various tissues, including ovary, testis, adipose tissue, placenta, liver, brain and bone (Simpson et al., 1994; Harada et al., 2003; Yanase et al., 2003; Stocco, 2008;

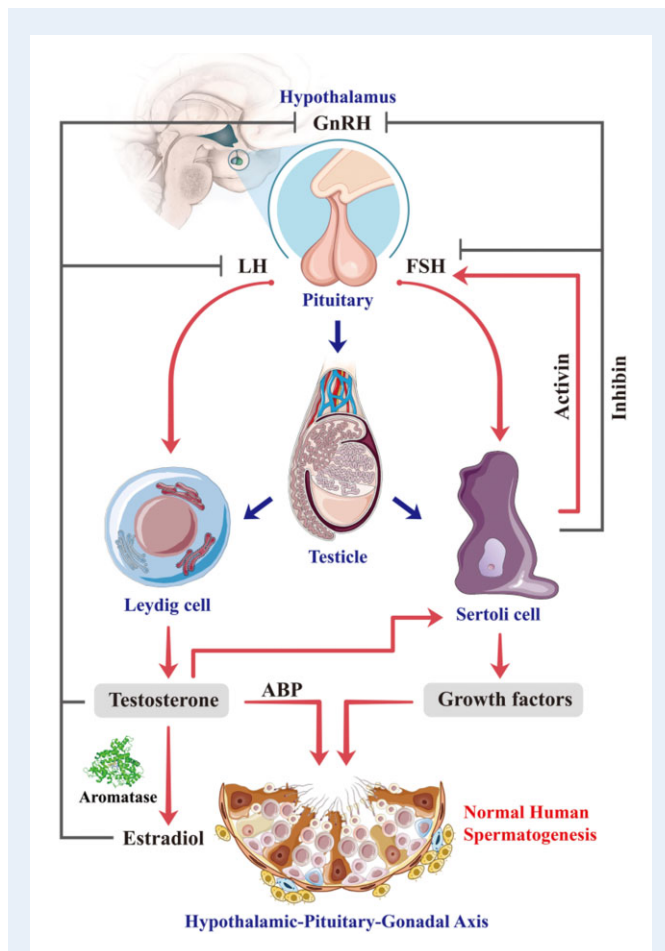


Figure 1. The hypothalamic–pituitary–gonadal axis in the male. GnRH is secreted from the hypothalamus, which stimulates anterior portion of the pituitary gland to produce LH and FSH. LH stimulates Leydig cell production of testosterone, and a fraction of testosterone is converted into estradiol by aromatase. FSH stimulates Sertoli cell to produce androgen-binding protein and various growth factors, which are essential to sustain spermatogenesis. Testosterone, estradiol and inhibin that secreted by Sertoli cell all generate negative feedbacks on hypothalamus and pituitary.

Murakami *et al.*, 2018; Brooks *et al.*, 2020; Kalicinska *et al.*, 2020). In the testis, aromatase is expressed in Leydig cells, Sertoli cells and germ cells such as spermatocytes, spermatids and spermatozoa. Aromatase is also expressed in the epididymis (Berensztejn *et al.*, 2006; Carreau *et al.*, 2010).

Aromatase is encoded by the *CYP19A1* gene, which is located on chromosome 15q21.2 and has a complex structure. It spans 123 kb, 30 kb of which contains the coding exons, exons 2–10. The remaining 93 kb region contains 10 tissue-specific noncoding upstream exons with separate promoters that regulate transcription in different cells and tissues (Sebastian and Bulun, 2001; Munro, 2018).

In men, aromatase is responsible for the conversion of C19 androgens androst-4-ene-3,17-dione (androstenedione) and T to the C18 estrogens, namely estrone and E₂, respectively (Osawa *et al.*, 1987;

Chen *et al.*, 1993). Strikingly, most bioavailable E₂ in the male is primarily created through peripheral aromatization of circulating T in adipose tissue (Nelson and Bulun, 2001; Simpson *et al.*, 2002).

The pathogenic effects of aberrant aromatase function in male

As aromatase is essential for maintaining the balance between androgens and estrogens, both aromatase deficiency and excess could result in many development disorders and clinical symptoms that depend on sex hormones.

Aromatase deficiency is a rare autosomal recessive disorder in which individuals cannot synthesize endogenous estrogens. Males with aromatase deficiency do not present with obvious defects at birth. Their clinical symptoms include tall stature, delayed skeletal maturation, delayed epiphyseal closure, bone pain, eunuchoid body proportions and excess adiposity (Robertson *et al.*, 1999; Lin *et al.*, 2007; Rochira and Carani, 2009).

Aromatase over-activity is characterized by increased aromatization of androgen that presents with symptoms of excess estrogen and reduced androgen, including hypogonadism, gynecomastia, premature growth spurt, early fusion of epiphyses and decreased adult height (Fukami *et al.*, 2012; Narula and Carlson, 2014; Almeida *et al.*, 2017). A few of these patients had a rare autosomal dominant disorder called aromatase excess syndrome (Fukami *et al.*, 2014; Shozu *et al.*, 2014). Notably, increased E₂ levels resulting from excess activity of aromatase has been observed in a proportion of infertile men, especially patients with NOA or oligozoospermia (Pavlovich *et al.*, 2001). In addition, excess aromatase activity is also seen in many obese men, as this enzyme is abundantly expressed in adipose tissues. These obese patients showed higher rates of hypogonadism, reduced sperm production and infertility (Sermondade *et al.*, 2013; Stephens and Polotsky, 2013).

More recent findings have also demonstrated that polymorphisms of the aromatase gene *CYP19A1* may be related to altered aromatase activity and sex hormone profiles, which may affect male fertility and treatment efficacy of AIs (Hammoud *et al.*, 2010a,b).

Requirement for estrogen in the male reproductive system

The functions of estrogen have been extensively studied, mainly in females. Nevertheless, the presence of estrogen in males has been known for over 90 years (Hess and Cooke, 2018). In males, estrogen mainly comes from the aromatization of androgen in the adipose tissues and testes. Three major endogenous estrogens have estrogenic activity including estrone (E₁), E₂ and estriol (E₃), with E₂ being the most potent and prevalent hormone (Shoham and Schachter, 1996; Carreau *et al.*, 2010). Previous studies have confirmed the essential roles of estrogen in male fertility based on the following key findings: first, the level of estrogen, especially E₂, within the male reproductive tract is far higher than that in the serum; second, testicular cells and the other parts of the male genital tract express at least two estrogen receptors (ER, including ER α and ER β), mutations of which have been observed in many infertile men; third, knockout animals revealed that ER is essential for male fertility and the development of efferent ductules, epididymis and prostate; fourth, animal experiments have shown a requirement for E₂ in sperm motility, capacitation, acrosome reaction and fertilizing capacity (Li *et al.*, 1997; Shetty *et al.*, 1997; Saunders *et al.*, 2001; Carreau *et al.*, 2011; Guido *et al.*, 2011; Eyster, 2016).

Detrimental effects of excess estrogen on spermatogenesis

Although estrogen is required for normal male fertility, excess estrogen, especially E_2 , has negative effects on the normal functions of testicular cells and spermatogenesis (Bharti et al., 2013). Clinical studies have identified an association between severely impaired spermatogenesis and high E_2 to T ratio in many infertile men, which suggested a cut-point of 10 as the lower limit of a normal T: E_2 ratio in men (units of T and E_2 are ng/dl and pg/ml, respectively) (Pavlovich et al., 2001). Compared with the serum level of T or E_2 alone, the ratio between the two hormones is more valuable and reliable for the evaluation of infertile men. In addition, as mentioned above, excess E_2 could exert a more powerful negative feedback on the HPG axis, resulting in decreased serum FSH and LH levels (Raven et al., 2006).

Previous studies also demonstrated detrimental effects of excess E_2 on Leydig cells using aromatase gain-of-function mice models, which were hypo-androgenic and hyper-estrogenic. These mice presented cryptorchidism and infertility. Testis pathological analysis revealed macrophages accumulation, Leydig cell hypertrophy and hyperplasia, and severely impaired spermatogenesis characterized by atrophic seminiferous tubules with only Sertoli cells remaining (Li et al., 2001, 2006, 2015). E_2 or an increased E_2 :T ratio not only promoted testicular macrophage activation but also regulated production of growth arrest-specific 6 (GAS6) and induces phosphatidylserine (PS) exposure on the surface of Leydig cells in an $ER\alpha$ -dependent manner. The subsequent interactions between GAS6, Leydig cells and testicular macrophages resulted in the formation of an AXL–GAS6–PS complex that led to Leydig cell engulfment, disrupted spermatogenesis and male infertility (Hutson, 1998; Hales, 2002; Hedger, 2002). Microarray analysis using human testes showed significantly enhanced expression of CYP19A1, GAS6 and AXL receptor tyrosine kinase in patients with NOA, compared to patients with OA and normal spermatogenesis, confirming the results obtained from mice model studies (Yu et al., 2014).

Owing to its powerful suppression on testicular function, estrogen has been extensively used in gender affirming treatment, especially the more potent E_2 . The goal for transgender females is to induce feminizing changes and suppress previously developed masculinization, and estrogen in sufficient doses will usually achieve both goals with augmentation by antiandrogens (Randolph, 2018; Radix, 2019). Specific effects of estrogen on the transgender female reproductive system include lower sperm count and quality, smaller prostate size, decreased penile erections, lower serum T, lower FSH and LH, augmented breast tissue etc. (Hembree et al., 2017; Tangpricha and den Heijer, 2017).

Clinical trials of aromatase inhibitors in treating male infertility

Als block the action of P450arom, thereby increasing endogenous T levels and reducing serum E_2 levels. A lower E_2 level relieves the excessive negative feedback on the HPG axis, resulting in increased T, FSH and LH production, which may improve spermatogenesis in infertile men (Turkistani and Marsh, 2012). Also, normalized E_2 could restore Leydig cell functions to support spermatogenesis.

To date, three types of Als have been empirically used for infertile men, including steroidal testolactone, and nonsteroidal anastrozole and letrozole, with the latter two commonly used at present (Serralini and

Moslemi, 2001). Many clinical trials have explored and reported on the treatment efficacy of Als in infertile patients, including azoospermic and oligozoospermic men. Some trials only included patients with a low T: E_2 ratio, while others had no specific selection. Thus, treatment results varied by patient population and indication for therapy. Sex hormones and semen parameters were evaluated in most trials. Results were compared among patients with comparable baseline characteristics. Hormonal changes, such as increases in serum T and FSH levels, were identified in most studies. Re-appearance of sperm in the ejaculate in patients with NOA and natural pregnancy were study endpoints. However, most trials were prospective cohort studies, retrospective cohort studies or case report/series, and randomized controlled trials (RCTs) were limited. The designs and results of these studies are summarized in Table I.

The studies summarized in Table I evaluated single-drug therapy. Other studies have evaluated a combination therapy of Als with additional agents, such as testosterone, to treat male infertility or hypogonadism, and these are summarized in Table II.

Here we have summarized 17 clinical trials conducted in the last 40 years that treated male infertility with Als, including two RCTs, one randomized equivalence trial, nine prospective cohort studies, two retrospective studies and three case report/series. Also, three trials that combined Als with other drugs for male infertility are also summarized.

Before 2002, only testolactone was used and tested, and most patients recruited were oligozoospermic. Most prospective studies showed significant improvement in hormone profiles (decreased E_2 , increased T and increased T: E_2 ratio) and sperm density after testolactone treatment, however, the only RCT of testolactone did not present the same results. In this study, 25 oligozoospermic patients were randomized to the testolactone or placebo group, and received drugs daily for 8 months, followed by crossover to the other treatment for another 8 months. Although FSH and LH increased significantly, both free and total E_2 , as well as semen parameters showed no significant changes, and no natural pregnancy occurred (Clark and Sherins, 1989). This RCT obviously challenged the application of testolactone in treating oligozoospermia and male infertility, and testolactone was superseded later by the non-steroidal Als letrozole and anastrozole.

Both letrozole and anastrozole were widely used for infertile men, including azoospermic, oligozoospermic and cryptozoospermic patients with normal or low T: E_2 ratio. In most prospective and retrospective studies, letrozole and anastrozole significantly elevated serum T level, T: E_2 ratio, serum FSH and LH levels, and sperm density after at least 3 months of treatment. Some azoospermic patients even had sperm in their ejaculates after treatment (Cavallini et al., 2011; Saylam et al., 2011; Kyrou et al., 2014). Unlike testolactone, the RCT further validated the treatment efficacy of letrozole for infertile men (Cavallini et al., 2013). Strikingly, patients with Klinefelter syndrome, patients with a former negative testis biopsy and patients with an adverse reaction to clomiphene citrate could also benefit from letrozole and anastrozole treatment (Raman and Schlegel, 2002; Kyrou et al., 2014; Shoshany et al., 2017). In addition, it was shown that combination of Als with T or clomiphene citrate may provide synergistic effects for hypoandrogenic infertile or subfertile men (Mehta et al., 2013; Mechlin et al., 2014; Alder et al., 2018). However, there are no studies comparing the treatment efficacy between combined drug therapy and single drug therapy. These results were consistent with a previous

Table 1 Clinical research using aromatase inhibitors to treat male infertility.

Reference	Research type	Patient number and characteristics	Treatment groups	Dose and duration	Study endpoints	Results	Comments
Vigersky and Glass (1981)	Prospective cohort study	10 normogonadotropic oligozoospermic infertile men; biopsies showed various abnormalities from normal spermatogenesis to variable degrees of maturation arrest.	Delta 1-testolactone	1 g/day; thrice daily; 6–12 months; orally	Serum E ₂ Serum T T:E ₂ ratio Serum FSH Serum LH Sperm density Sperm count Sperm motility Semen volume Pregnancy Testis volume	SD ($P < 0.01$) SI ($P < 0.02$) SI ($P < 0.01$) NS NS SI ($P < 0.01$) SI ($P < 0.001$) NS NS Three patients NS	Testolactone had no significant effects on FSH and LH, but increased T, T:E ₂ ratio, sperm density and count. No obvious side-effects were seen despite the long period of treatment
Dony et al. (1986)	Prospective cohort study	Nine normogonadotropic oligozoospermic infertile men.	Delta 1-testolactone	500 mg; twice daily; 6 months; Orally	Serum FSH Serum LH Serum E ₂ Serum total T Serum SHBG T:SHBG ratio T:E ₂ ratio Sperm density Sperm count Percentage of abnormal forms Sperm motility Pregnancy rate	SI ($P < 0.05$) NS SD ($P < 0.01$) SI ($P < 0.05$) SD ($P < 0.005$) SI ($P < 0.025$) SI ($P < 0.05$) SI ($P < 0.01$) SI ($P < 0.05$) SD ($P < 0.025$) NS 2/9	Testolactone reduced both E ₂ and SHBG levels, and increased FSH, T, and T:E ₂ ratio, with no effects on LH. Sperm counts rose and abnormal forms of sperms decreased.
Clark and Sherins (1989)	Randomized controlled double-blind crossover trial	25 infertile patients with unexplained oligozoospermia.	1. Testolactone 2. Placebo (cornstarch)	2 g/day; 8 months and followed by crossover to the other treatment for 8 months; Orally	Serum total E ₂ Serum total T Serum LH Serum FSH SHBG binding capacity Free E ₂ Free T Total sperm counts Sperm density Sperm motility Morphology Pregnancy rate	NS NS SI (15%, $P < 0.05$) SI (20%, $P < 0.05$) SD (30%, $P < 0.01$) NS SI (36%, $P < 0.01$) NS, though seven men increased by more than 2-fold NS NS NS NS None	The first RCT of AI in treating male infertility. Although T, FSH, LH increased significantly, both free and total E ₂ , as well as semen parameters showed no significant changes. Patients who are not hypoandrogenic may not benefit a lot from AI treatment.
Itoh et al. (1991)	Prospective cohort study	Nine infertile men with oligozoospermia and a high E ₂ :T ratio.	Testolactone	1.0 g/day; 3 months; Orally	Sperm density Sperm motility Serum E ₂ E ₂ :total T ratio E ₂ :free T ratio Serum total T Serum free T Serum SHBG Serum LH Serum FSH	NS SI ($P < 0.05$) SD ($P < 0.01$) SD ($P < 0.01$) SD ($P < 0.01$) NS SI ($P < 0.01$) SD ($P < 0.01$) NS NS	The first trial focused on patients with a high E ₂ :T ratio. E ₂ , E ₂ :T ratio, and SHBG all decreased after treatment, but sperm density, LH and FSH showed no significant changes.
Pavlovich et al. (2001)	Prospective cohort study	63 infertile men, 43 were azoospermic, 20 were oligozoospermic, 45 of them had a low T:E ₂ ratio.	Testolactone	50–100 mg; twice daily; 5 months; Orally	Serum E ₂ Serum T T:E ₂ ratio Serum LH Serum FSH Sperm density Sperm motility Total sperm per ejaculate	SD ($P < 0.01$) SI ($P < 0.01$) SI ($P < 0.01$) NS Not available Oligozoospermic: SI ($P = 0.03$) Azoospermic: None Oligozoospermic: SI ($P < 0.01$) Azoospermic: None Oligospermic: NS Azoospermic: None	The first trial included both oligozoospermic and azoospermic men with low T:E ₂ ratio. E ₂ and E ₂ :T ratio reduced after treatment, with improved semen parameters only in oligozoospermic men. LH and FSH had no significant changes.

Continued

Table I Continued

Reference	Research type	Patient number and characteristics	Treatment groups	Dose and duration	Study endpoints	Results	Comments
Raman and Schlegel (2002)	Prospective cohort study	140 subfertile men who had abnormal T:E ₂ ratio, including patients with Klinefelter's syndrome, obesity and varicocele.	1. Testolactone (74 patients) 2. Anastrozole (104 patients)	1. Testolactone 50–100 mg twice daily; 6 months; orally 2. Anastrozole 1 mg/day; 4.7 months; orally	Serum T Serum E ₂ T:E ₂ ratio Sperm density Morphology Sperm motility Semen volume	NS 1. Not available 2. SD ($P < 0.05$) SI ($P < 0.001$) SI ($P < 0.05$) 1. SI ($P < 0.05$) 2. Not available 1. SI ($P < 0.05$) 2. SI ($P < 0.005$) 1. Not available 2. SI ($P < 0.05$)	Patients all had a low T:E ₂ ratio and many had specific causes. Both testolactone and anastrozole increased T:E ₂ ratio, sperm density and motility, but treatment efficacy was not compared between them.
Patry et al. (2009)	Case study	A primary azoospermic man with normal FSH level, proven to be hypospermatogenesis (HS) by biopsy.	Letrozole	2.5 mg/day; 3 months; orally	Serum LH Serum FSH Serum T Serum E ₂ Testis biopsy	Increased from 3 to 35 IU/l Increased from 2 to 11 IU/l Increased from 14 to 28 nmol/l Not available Improved from HS to normal	The first case using biopsy to evaluate the treatment efficacy of AI, which improved from HS to normal.
Cavalliniet al. (2011)	Prospective cohort study	Four azoospermic men with normal sex hormones.	Letrozole	2.5 mg/day; 3 months; orally	Serum FSH Serum LH Serum T Serum E ₂ Serum PRL T:E ₂ ratio Sperm density	SI ($P < 0.05$) SI ($P < 0.05$) SI ($P < 0.05$) SI ($P < 0.01$) SD ($P < 0.01$) SI ($P < 0.05$) Sperms appeared in all patients	The first trial reported recurrence of sperms in azoospermic patient after AI treatment.
Saylam et al. (2011)	Prospective cohort study	27 idiopathic infertile men with a low T:E ₂ ratio (< 10). 10 are oligozoospermic 17 are azoospermic	Letrozole	2.5 mg/day; 6 months; orally	Sperm density Sperm motility Semen volume FSH Serum T Serum E ₂ T:E ₂ ratio Pregnancy rate Sperm occurrence BMI Testis volume	SI ($P = 0.002$) SI ($P = 0.017$) SI ($P < 0.001$) NS SI ($P < 0.001$) SD ($P < 0.001$) SI ($P < 0.001$) 2/27 (2 oligozoospermic patients) 4/17 (4 azoospermic patients had sperms in the semen) NS NS	Both oligozoospermic and azoospermic men benefited from the letrozole. Hormone profiles and semen parameters improved except for FSH. Strikingly, 4 out of 17 azoospermic patients showed sperms in the semen after 6-months of treatment.
Gregoriou et al. (2012)	Prospective Cohort study	29 infertile men with a serum T:E ₂ < 10 , T levels < 300 ng/dl and sperm density $< 10 \times 10^6$ /ml	1. Letrozole (15 patients) 2. Anastrozole (14 patients)	1. Letrozole 2.5 mg/day; 6 months; orally 2. Anastrozole 1 mg/day; 6 months; orally	Serum FSH Serum LH Serum T Serum E ₂ T:E ₂ ratio Ejaculate volume Total sperm count Sperm motility Total functional sperm fraction	NS in both groups NS in both groups SI in both groups ($P < 0.001$) SD in both groups ($P < 0.001$) SI in both groups ($P < 0.001$) 1. SI ($P = 0.005$) 2. SI ($P < 0.001$) 1. SI ($P = 0.001$) 2. SI ($P < 0.001$) 1. SI ($P = 0.001$) 2. SI ($P < 0.001$) 1. SI ($P = 0.013$) 2. SI ($P = 0.005$)	Both anastrozole and letrozole significantly improved hormone profiles and semen parameters. However, treatment efficacy was not compared between the two drugs.
Cavallini et al. (2013)	Randomized controlled study	11 NOA patients and 35 cryptozoospermic patients, all of them had a T:E ₂ ratio < 10 .	1. Letrozole 6 azoospermic 16 cryptozoospermic 2. Placebo 5 azoospermic 19	1. Letrozole 2.5 mg/day; 6 months; Orally 2. Placebo 100 mg/day;	Serum FSH Serum LH Serum T Serum E ₂ Serum PRL Total sperm count	1. SI ($P < 0.01$) 2. NS 1. SI ($P < 0.01$) 2. NS 1. SI ($P < 0.01$) 2. NS	The first RCT of Als in treating infertile men with low T:E ₂ ratio, which showed letrozole significantly improved the hormone

Continued

Table I Continued

Reference	Research type	Patient number and characteristics	Treatment groups	Dose and duration	Study endpoints	Results	Comments
Cavallini <i>et al.</i> (2013)			cryptozoospermic	6 months; Orally	Sperm motility	1. SD ($P < 0.01$) 2. NS 1. NS 2. NS 1. SI ($P < 0.01$) 2. NS 1. SI ($P < 0.01$) 2. NS	profiles, and increased sperm counts and motility.
Zhao <i>et al.</i> (2014)	Case study	One azoospermic man with small testis, normal T and E ₂ , and an elevated FSH level, diagnosed as HS by testicular biopsy.	Letrozole	2.5 mg/day; 3 months; Orally	Serum LH Serum FSH Serum T Serum E ₂ Serum PRL Semen analysis	Rose from 6.80 to 13.49 mIU/ml Rose from 29.38 to 49.15 mIU/ml Rose from 4.23 to 10.57 ng/ml Decreased from 36 to 12 pg/ml Rose from 17.30 to 22.70 mIU/ml Sperm density rose to 20.92×10^6 /ml, with a 3.87% motility	The first successful case using letrozole to treat NOA patient with elevated FSH level.
(Kyrrou <i>et al.</i> 2014)	Case series	2 azoospermic patients with low T and normal FSH; biopsies showed that patient 1 (P1) was maturation arrest, and patient 2 (P2) was SCOS.	Letrozole	2.5 mg/48 h; 4 months; Orally	Serum T TESE Sperm density Progressive motility	P1: Rose from 3.3 to 10 nmol/l P2: Rose from 6.4 to 23.5 nmol/l Sperms were found in both cases P1: 0.016 mill/ml P2: 0.023 mill/ml P1: 36% P2: 22%	Even patients of maturation arrest or SCOS could benefit from AI, indicating limitations of testis biopsy in guiding the treatment of NOA.
Helo <i>et al.</i> (2015)	Prospective double-blind randomized equivalence trial	26 hypogonadal infertile male with serum T level less than 350 ng/dl and normal LH.	1. Anastrozole (AZ) (13 patients) 2. Clomiphene citrate (CC) (13 patients)	1. AZ 1 mg/day; 12 weeks; Orally 2. CC 25 mg/day; 12 weeks; Orally	Serum T T:E ₂ ratio Serum E ₂ Serum LH Serum FSH Serum SHBG Sperm density Sperm motility	1. SI ($P < 0.05$) 2. SI ($P < 0.05$) 1. SI ($P < 0.05$) 2. NS 1. Decreased 2. Increased 1. SI ($P < 0.05$) 2. SI ($P < 0.05$) 1. Increased 2. Decreased 1. NS 2. SI ($P < 0.05$) NS was noted in both groups or between the two groups. NS was noted in both groups or between the two groups.	The first study that compared the effect of AI and SERM. AZ induced a higher T:E ₂ ratio, however, both AZ and CC were comparable on FSH, LH and semen quality of infertile male with low serum T level and normal LH. Patients with abundant sperms may not benefit a lot from AI treatment.
Shoshany <i>et al.</i> (2017)	Retrospective study	86 hypoandrogenic sub-fertile men with low T:E ₂ ratio (78) or a prior adverse reaction to clomiphene citrate (8).	Anastrozole	1 mg/day; 4 months; Orally	Serum total T Free T Serum E ₂ T:E ₂ ratio Serum LH Serum FSH Semen volume Sperm density Sperm motility Motile sperms	SI ($P < 0.0001$) SI ($P < 0.0001$) SD ($P < 0.0001$) SI ($P < 0.0001$) SI ($P < 0.0001$) SI ($P < 0.0001$) NS SI ($P < 0.001$) NS SI ($P < 0.01$)	Nearly all patients had improved hormone profile, however, only oligozoospermic men had improved semen parameters.

Continued

Table I Continued

Reference	Research type	Patient number and characteristics	Treatment groups	Dose and duration	Study endpoints	Results	Comments
Shuling et al. (2019)	Prospective study	15 idiopathic severe oligozoospermic (sperm density < 5×10^6 /ml) patients with normal T:E ₂ ratio.	Letrozole	2.5 mg/day; 4 months; Orally	Serum T Serum E ₂ T:E ₂ ratio FSH LH Sperm density Sperm count Sperm motility	SI (2.5-fold, $P < 0.0001$) SD (0.6-fold, $P = 0.0033$) SI ($P < 0.0001$) SI (2.1-fold; $P = 0.0005$) SI (2.2-fold; $P < 0.0001$) SI (5.5-fold, $P = 0.0068$) SI (4.3-fold, $P = 0.0096$) NS	Oligozoospermic men with normal T:E ₂ ratio got higher serum T and T:E ₂ ratio, as well as increased sperm counts after letrozole treatment.
Peivandi et al. (2019)	Retrospective study	41 infertile men with a T:E ₂ ratio < 10.	Letrozole	2.5 mg/day; 4 months; Orally	Serum T Serum E ₂ T:E ₂ ratio FSH LH Sperm density Sperm motility Forward sperm	SI ($P < 0.001$) SD ($P = 0.002$) SI ($P = 0.014$) SI ($P < 0.001$) SI ($P < 0.001$) SI ($P < 0.001$) SI ($P < 0.001$) SI ($P < 0.001$)	Letrozole improved both hormone profile and semen parameters in oligozoospermic and asthenospermic patients with low T:E ₂ ratio, including sperm motility.

SI, significant increase; SD, significant decrease; NS, no significant difference; AI, aromatase inhibitor; HS, hypospermatogenesis; SCOS, Sertoli cell only syndrome; AZ, anastrozole; TESE, testicular sperm extraction; T, testosterone; E₂, estradiol; SHBG, sex hormone-binding globulin; NOA, non-obstructive azoospermia.

systematic review and meta-analysis on this topic (Del Giudice et al., 2020): the authors evaluated eight original articles on application of AIs to infertile/hypoandrogenic males and found that either steroidal (testolactone) or nonsteroidal (anastrozole and letrozole) AIs significantly improved hormone profile and semen parameters within tolerable safety limits. It is worth noting that some subjects gained little or no benefit from AIs treatment, and the biological explanation for this remains elusive, indicating that further basic and clinical research is required.

Aromatase inhibitors for obesity-related male infertility and hypogonadism

Impact of obesity on male fertility and potential mechanisms

Spermatogenesis is a process that is highly dependent on the intact HPG axis. Of all the factors that disturb this axis, obesity has been relatively less well studied in the setting of male infertility. The incidence of obesity is increasing rapidly worldwide, which is associated with various diseases such as type II diabetes, coronary artery diseases, hypertension and impaired reproductive health (Maggio and Pi-Sunyer, 2003; Sallmen et al., 2006; Seravalle and Grassi, 2017; Csige et al., 2018; Lainez and Coss, 2019). In women, the negative impact of obesity on reproductive function is readily evident, including female subfertility and obstetric complications (Pasquali et al., 2003; Ramlau-Hansen et al., 2007; Satpathy et al., 2008; Broughton and Moley, 2017). For men, an interesting fact is that the global obesity rate has increased while sperm quality has declined (Carlsen et al., 1992; Ng et al., 2014). Recent population-based studies showed an elevated risk for subfertility and an increased likelihood of abnormal semen parameters, unbalanced reproductive endocrine function and attenuated sexual function among men with a high BMI. In addition, emerging evidence

demonstrated that obesity perturbs the physical and molecular structure of germ cells and ultimately affects the maturity and function of sperm (Hofny et al., 2010; Teerds et al., 2011; Palmer et al., 2012). Many investigators have attributed sperm decline to the contemporaneous global rise of obesity. Table III lists several representative large-scale studies evaluating the effects of obesity on human male fertility.

Among these studies, consistent findings included significantly decreased sex hormone-binding globulin and T levels, increased E₂ level and a subsequent decreased T:E₂ ratio in obese men. No significant differences in FSH and LH levels were identified between normal and obese men. Reduced semen quality was observed in most studies. A meta-analysis demonstrated that a J-shaped relation exists between BMI categories and risk of oligozoospermia or azoospermia. Compared with men of normal weight, the odds ratio (95% CI) for oligozoospermia or azoospermia was 1.15 (0.93–1.43) for underweight, 1.11 (1.01–1.21) for overweight, 1.28 (1.06–1.55) for obese and 2.04 (1.59–2.62) for morbidly obese men (Sermondade et al., 2013). However, only a few studies found an association between BMI and reduced sperm motility or morphology (Jensen et al., 2004; Kort et al., 2006; Hammoud et al., 2008).

The pathogenic mechanisms underlying impaired fertility in obese men are complex, with increased peripheral aromatization of androgens as the pivotal etiological factor. It is known that aromatase is highly expressed in adipose tissue and it has been observed that aromatase activity is increased in obese men, which parallels the decreased serum T level, and increased estrone and E₂ levels (Kley et al., 1980). Apart from aromatase, insulin resistance, sleep apnea and cardiovascular diseases have also been proposed to play important roles in the morbidity of obesity-related male infertility and hypogonadism. The relations of these etiological factors to male infertility are

Table II Clinical trials combining aromatase inhibitors with other drugs to treat male infertility and hypogonadism.

Reference	Research type	Patients' condition and numbers	Treatment groups	Dose and duration	Study endpoints	Results	Comments
Mehta et al. (2013)	Retrospective study	10 azoospermic patients proven to be Klinefelter syndrome, and all of them had a T:E ₂ ratio <10.	Testosterone (T) plus anastrozole (AZ)	T: 2.5–10 g/day; for patients with T < 350 ng/dl. AZ: 1 mg/day; orally; 6–24 months; for all patients.	Serum T Serum LH Serum FSH Micro-TESE	Rose from 192 to 600 ng/dl Rose from 8.5 to 14.8 mIU/ml Rose from 18.5 to 33.5 mIU/ml Sperms were retrieved in seven patients, and sperm density ranged from 0.1 to 2 × 10 ⁶ /ml	Patients of Klinefelter syndrome benefited from combination of T and AI.
Mechlin et al. (2014)	Retrospective study	65 symptomatic hypogonadal men (T < 350 ng/dl) previously treated with testosterone pellet (TP).	TP (34 patients) TP + AZ (31 patients)	TP: 10 pellets (75 mg per pellet) TP: 10 pellets; AZ: 1 mg/day, orally	Serum total T Serum free T Serum E ₂ T:E ₂ ratio Serum LH Serum FSH Serum SHBG Reinsertion time	SI in TP + AZ group (<i>P</i> < 0.05) SI in TP + AZ group (<i>P</i> < 0.01) SD in TP + AZ group (<i>P</i> < 0.05) SI in TP + AZ group (<i>P</i> < 0.01) SI in TP + AZ group (<i>P</i> < 0.05) SI in TP + AZ group (<i>P</i> < 0.01) NS AZ increased time of TP reinsertion (<i>P</i> < 0.01)	AI could reinforce the treatment efficacy of T in improving hormone profiles of patients with hypogonadism, while its effects on semen parameters were not evaluated.
(Alder et al. 2018)	Retrospective study	51 hypoandrogenic subfertile men, who had received clomiphene citrate (CC) treatment for about 2.3 months.	CC + AZ	AZ was added to CC in patients with E ₂ > 50 pg/ml or T:E ₂ ratio < 10; 1 mg; twice to thrice weekly for average 8 months.	Serum total T Serum free T Serum E ₂ T:E ₂ ratio Sperm motility Sperm density	NS NS SD (<i>P</i> < 0.001) SI (<i>P</i> < 0.001) NS SI (<i>P</i> = 0.03). 2 oligozoospermic patients became normal; 4 azoospermic patients had sperms.	AZ could reduce high E ₂ level caused by CC treatment for subfertile men, and improve sperm counts. AI and SERM could have synergistic effects.

TP, testosterone pellet; AZ, anastrozole; CC, clomiphene citrate; AI, aromatase inhibitor; micro-TESE, microdissection testicular sperm extraction.

summarized in [Fig. 2](#) ([Stellato et al., 2000](#); [Valencia-Flores et al., 2000](#); [Luboshitzky et al., 2005](#); [O'Brien et al., 2005](#)).

Clinical trials of aromatase inhibitors in treating obesity-related male infertility and hypogonadism

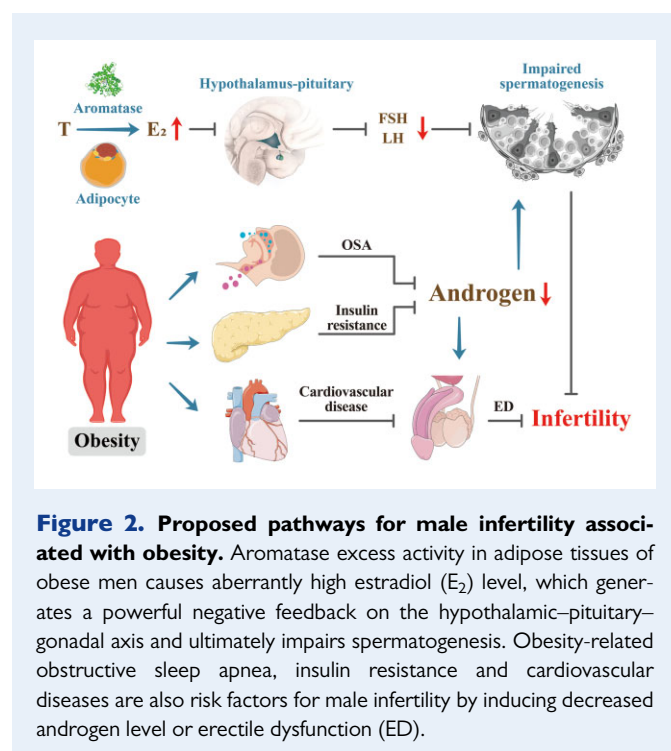
The altered hormone profile in obese men suppresses spermatogenesis and induces hypogonadal symptoms via inhibition of the HPG axis. These patients may benefit from the direct inhibition of E₂ synthesis by using AIs. Many studies have evaluated the therapeutic effects of AIs in treating obesity-related male infertility and hypogonadism, and these are summarized in [Table IV](#).

In these studies, nearly all subjects achieved an improved hormone profile after 6 months of AIs treatment, especially a decreased serum E₂ level, increased free T level and increased T:E₂ ratio. The partner of a patient with oligozoospermia and obesity who underwent 6 months of anastrozole treatment even achieved a natural pregnancy ([Roth et al., 2008](#)). The only RCT demonstrated a significant increase of FSH and LH, which was not seen in other case series ([Loves et al., 2013](#)). Unfortunately, whether AIs can improve spermatogenesis in obese men remains unknown, as only one case report observed changes in semen parameters ([Roth et al., 2008](#)).

Table III Population-based studies on the impacts of obesity on male fertility.

Reference	Research type	Subjects	Groups (BMI (kg/m ²))	Outcome measures	Results
Jensen et al. (2004)	Cross-sectional study	1558 young men attending a physical examination.	Underweight: <20; Normal: 20–25; Overweight: >25	Sperm density Total sperm count Normal form of sperms Testis size Semen volume Motile sperm Sex hormones	Lower in underweight and overweight groups by 28.1% and 21.6%. Lower in underweight and overweight groups by 36.4% and 23.9%. Lower in underweight and overweight groups with no significance. Smaller in underweight group. Lower in underweight group. Lower in underweight group. With increasing BMI, T, SHBG and inhibin B decreased, whereas androgen and E ₂ increased significantly.
Kort et al. (2006)	Retrospective cohort analysis	520 healthy men presenting for semen analysis.	Normal: 20–24; Overweight: 25–30; Obese: >30	NMS DFI	Negatively related to BMI. Significant difference among three groups. Positively related to BMI. Significant difference between normal group and overweight or obese group.
Aggerholm et al. (2008)	Cross-sectional study	2139 men that participated studies of semen quality.	Underweight: <20; Normal: 20–25; Overweight: 25.1–30; Obese: >30	Sperm density Total sperm count Sperm motility Sex hormones	NS among four groups. NS among four groups. NS among four groups. Inhibin B, SHBG and T significantly decreased with increasing BMI.
Hammoud et al. (2008)	Retrospective cohort analysis	390 couples presenting for infertility evaluation, who had no known factor that affects fertility.	Normal: <25 (94, 24.1%); Overweight: 25–30 (168, 43.1%); Obese: >30 (128, 32.8%)	Incidence of oligozoospermia OR of oligozoospermia Low progressively motile sperm count OR of abnormal morphology rate Incidence ED	Increased significantly with BMI. OR in obese group compared with normal was 3.3. Increased significantly with BMI. OR of having high abnormal morphology rate in obese patients compared with non-obese patients was 1.6. Increased with increasing BMI with no significant difference.
Chavarro et al. (2010)	Cross-sectional study	483 male partners of subfertile couples attending an infertility clinic	Normal: 18.5–24.9; Overweight: 25–29.9; Obese: 30–34.9; Extremely obese: ≥35	Sperm density Sperm motility Sperm morphology Sperm count Progressive sperm Ejaculate volume Sperm DFI Sex Hormones	NS among four groups. NS among four groups. NS among four groups. Decreased significantly with increasing BMI. NS among four groups. Negatively related to BMI. Increased significantly with high DFI. BMI was negatively related to total T, SHBG, T/LH ratio and inhibin B, and positively related to E ₂ .

NMS, normal motile sperm; DFI, DNA fragmentation index; OR, odds ratio; ED, erectile dysfunction.



Apart from published clinical trials, registered trials of using AIs to treat male infertility or hypogonadism were also reviewed and are summarized in Table V. Most of these studies were RCTs, focusing on symptomatic hypogonadal men with or without obesity. Medication included letrozole, anastrozole, and BGS649, a novel non-steroidal AI. Main outcome measures not only included reproductive parameters, such as sex hormones and hypogonadal symptoms, but also other parameters associated with testosterone, such as lipid metabolism, bone metabolism, muscle strength and prostate-specific antigen.

Relations between aromatase polymorphisms and male fertility

Overactivity of aromatase and the subsequent high E_2 level were proposed to contribute to the suppression of gonadotrophins, decreased testosterone production and impaired spermatogenesis. However, the mechanism involved in excessive aromatase activity remains unclear.

In recent years, aromatase polymorphisms were postulated to be implicated in impaired sperm production among men with excess aromatase activity. Actually, aromatase polymorphisms have been shown to be related to various estrogen-dependent diseases in both women and men, e.g. osteoporosis and breast cancer (Carreau *et al.*, 2010; Hammoud *et al.*, 2010b; Wang *et al.*, 2010). The most commonly studied aromatase polymorphisms include single nucleotide polymorphisms (SNPs) and the tetranucleotide TTTA repeat polymorphism (TTTAn) located in Intron 4 of the *CYP19A1* gene (Czajka-Oraniec *et al.*, 2008). TTTAn polymorphisms in men are postulated to affect aromatase activity, serum estrogen levels and sperm parameters (Hammoud *et al.*, 2010a,b), while SNPs were mainly studied in women with breast cancer, and the results demonstrated that *CYP19A1* SNPs had a close association with treatment efficacy of AIs (Ferraldeschi

et al., 2012; Artigas *et al.*, 2015). The findings of these studies are summarized in Table VI. As research into *CYP19A1* SNPs in male patients was limited, we also summarized relevant research conducted in female patients with breast cancer.

With respect to TTTAn polymorphisms, the number of TTTAn repeats in most men was lower than nine, and higher numbers of TTTAn repeats were associated with increased aromatase activity, presenting as an elevated circulating E_2 level, which paralleled the decrease in sperm concentration. The higher circulating E_2 level also led to lower FSH levels in men with high-repeat numbers. In addition, in men with higher TTTA repeats, an increased BMI was associated with an exaggerated decrease in T: E_2 ratio and a reduction in sperm concentration. This may explain some heterogeneity of results among studies describing the effect of increased BMI on sex hormones and sperm parameters. It is plausible that the subjects of these studies had a different distribution of TTTAn genotypes, either enhancing aromatase activity among obese men with already high aromatase levels (higher proportion of men with high TTTA repeats) or blunting the effect of higher aromatase levels among obese men (higher proportion of men with low TTTA repeats). Treatment efficacy among men with different numbers of TTTA repeats may also vary; men with more TTTA repeats may require a higher dose or longer course of treatment with AIs. However, further research is necessary to better delineate this relation between *CYP19A1* polymorphisms and treatment efficacy of AIs for infertile or hypogonadal men.

SNPs of *CYP19A1* have been primarily studied in female patients with breast cancer, however, the results may provide some enlightenment on the evaluation and treatment of male infertility. Infertile men with poor response to AIs may have *CYP19A1* SNPs that affect aromatase activity and treatment efficacy. For these patients, *CYP19A1* SNPs analysis would be recommended, and alternative therapies are required.

Side-effects of aromatase inhibitors

Spermatogenesis is a relatively slow progress. Normally, it takes 64 days for spermatogonia stem cells to differentiate into sperm (Komeya and Ogawa, 2015). This fact determinates that drug therapy for male infertility should be stable and sustained. In this situation, the dose and duration of AIs are important considerations in terms of safety. In addition, application of AIs for male infertility treatment is off-label and empirical at present, and it is vital to pay close attention to the side-effects of AIs. Although no specific dose has been defined for infertile men, a common dose for anastrozole and letrozole is 1 and 2.5 mg/day, respectively. The treatment duration reported in most studies was at least 3 months, and no longer than 12 months. Although side-effects were inevitable, most subjects went through the trials with no malaise, and only a few dropped out. All side-effects of AIs in treating male infertility are summarized in Table VII.

In general, treating male infertility with AIs is well tolerated and safe for most patients. Among the side-effects, loss of libido was most common, especially for letrozole. Regular sexual intercourse is an indispensable part of male infertility treatment, therefore much attention should be paid to this side-effect. For patients who could not overcome declined sexual desire or sexual arousal, anastrozole may serve as a better alternative. In addition, about 10% of subjects presented increased liver enzymes during letrozole and anastrozole treatment, so it

Table IV Clinical studies of aromatase inhibitors in treating obesity-related male infertility and hypogonadism.

Reference	Research type	Patients' condition and numbers	BMI (kg/m ²)	Treatment groups	Dose and duration	Study endpoints	Results
Zumoff et al. (2003)	Case series	Six obese men with hypogonadotropic hypogonadism	49.3 ± 15.6	Testolactone	1 g/day; 6 weeks; orally	Serum E ₂ Serum T Serum LH Serum FSH	SD ($P < 0.004$) SI ($P < 0.0003$) SI ($P < 0.004$) N.S
de Boer et al. (2005)	Case series	10 severely morbid obese men with hypogonadotropic hypogonadism	42.1 ± 2.6	Letrozole	2.5 mg/day or 2.5 mg thrice a week; 6 months; orally	Serum E ₂ Serum LH Serum FSH Serum total T Serum SHBG T:E ₂ ratio	SD ($P = 0.006$) SI ($P < 0.001$) SI ($P < 0.001$) SI ($P < 0.001$) NS SI ($P < 0.005$)
Roth et al. (2008)	Case study	A 29-year-old obese man with infertility, hypogonadism, low sperm count, low T:E ₂ ratio and gynecomastia	54.5	Anastrozole	Dose was unclear; 6 months; orally	Serum E ₂ Serum total T Serum FSH Serum LH Sperm density Pregnancy	Declined (47 to < 20 pg/ml) Rose (5.3–14.7 nmol/l) Rose (2–34 IU/l) Rose (<1 to 9 IU/l) Rose (<2 to 21×10^6 /ml) Spontaneous pregnancy
Loves et al. (2008)	Case series	12 obese men with hypogonadotropic hypogonadism and free T levels <225 pmol/l	>35	Letrozole	2.5 mg once a week; 6 months; orally	Serum total E ₂ Serum free E ₂ Serum total T Serum free T Serum FSH Serum LH T:E ₂ ratio SHBG	SD ($P < 0.005$) SD ($P < 0.005$) SD ($P < 0.001$) SD ($P < 0.001$) SD ($P < 0.001$) SD ($P < 0.001$) SD ($P < 0.001$) NS
Loves et al. (2013)	RCT	42 obese men with hypogonadotropic hypogonadism and free T levels <10 nmol/l	>35	1. Letrozole 2. Placebo	Started on one tablet of 2.5 mg each week, added by one tablet every month; 6 months; orally	Serum LH Serum FSH Serum total T Serum free T Serum E ₂	1. SI ($P < 0.001$) 2. NS 1. SI ($P < 0.001$) 2. NS 1. SI ($P < 0.001$) 2. NS 1. SI ($P < 0.001$) 2. NS 1. SD ($P < 0.01$) 2. SD ($P < 0.01$)

SI, significant increase; SD, significant decrease; NS, no significant difference.

is necessary to monitor the patient's liver function carefully. In most trials, the investigators conducted a liver function test once a month for the subjects. The incidence of other side-effects was relatively low, such as rash, dry mouth, ocular symptoms and digestive system symptoms, but these side-effects affected the daily life of a few subjects and were more likely to reduce their compliance to the treatment, therefore the patients still need to be fully informed.

Conclusions and future perspectives

Als have been shown to effectively improve the sex hormone profile and semen quality in infertile men with or without a low serum T:E₂ ratio, and pregnancies may be achieved naturally after the treatment. Obesity is closely associated with hypogonadotropic hypogonadism

Table V Registered clinical trials of aromatase inhibitors in treating male infertility and hypogonadism.

Registration ID	Research type and status	Patients' condition and numbers	Inclusion criteria	Arms and interventions	Main outcome measures
NCT 02730169	RCT Phase II b Completed	271 obese men with hypogonadotropic hypogonadism	<ol style="list-style-type: none"> Adult men aged 18–65 years; 30 kg/m² < BMI < 50 kg/m²; Serum T below the normal range; LH levels below the upper limit of normal; E₂ levels within or above the normal range; At least two symptoms of androgen deficiency present for at least 2 months, including sexual dysfunction 	<ol style="list-style-type: none"> BGS649, 0.1 mg, PO, weekly BGS649, 0.3 mg, PO, weekly BGS649, 1.0 mg, PO, weekly Placebo, PO, weekly Duration: 24 weeks 	<ol style="list-style-type: none"> Percent of patients with normalized T level; Mean change from baseline in LH, FSH, PSA, hematocrit, DEXA scan density and score, bone turnover marker, bone specific ALP; BGS649 semen and plasma PK concentration
NCT 03490513	RCT Recruiting	120 obese men with hypogonadotropic hypogonadism	<ol style="list-style-type: none"> Obese men with BMI ≥ 35 kg/m²; Age between 40 and 65 years; Serum T < 300 ng/dl; LH < 9.0 mIU/l; E₂ ≥ 17 pg/ml; Symptoms of androgen deficiency 	<ol style="list-style-type: none"> Weight loss plus placebo, PO, daily Weight loss plus anastrozole, 1 mg, PO, daily Duration: 12 months 	<ol style="list-style-type: none"> Hormone profiles (E₂, LH, FSH); 2) muscle strength; 3) lean mass; 4) total hip bone mineral density; 5) thigh muscle volume; 6) symptoms of hypogonadism; 7) visceral adipose tissues; 8) metabolic risk factors; 9) bone density, quality, and markers
NCT 00104572	RCT Phase II Completed	44 older men with low testosterone	<ol style="list-style-type: none"> Men age 65 years or older; Serum T level ≤ 350 ng/dl; Subject is able to complete an informed consent 	<ol style="list-style-type: none"> T gel (1 mg) plus dietary supplement, daily Anastrozole (1 mg) plus dietary supplement, daily Placebo, daily Duration: 12 months 	<ol style="list-style-type: none"> Bone mineral density; Pulsatile growth hormone release; Glucose tolerance/lipid metabolism; Prostate volume, PSA level, urinary function
NCT 00136695	RCT Phase II Completed	100 elderly men with hypogonadism	<ol style="list-style-type: none"> Men ages 60 and older; Serum T between 150 and 300 ng/dl; Symptoms of androgen deficiency 	<ol style="list-style-type: none"> Anastrozole, 1 mg, PO, daily Placebo, 1 mg, PO, daily Duration: 24 months 	Lean body mass
NCT 00138710	RCT Phase III Completed	50 obese men with hypogonadotropic hypogonadism	<ol style="list-style-type: none"> BMI between 35 and 50 kg/m²; Male sex; Ages between 20 and 50 years; Serum total T under 10 nmol/l; Serum LH under 9 mIU/l; Serum E₂ over 40 pmol/l 	<ol style="list-style-type: none"> Letrozole, PO, daily Placebo, PO, daily Dose: adjusted according to serum T or E₂ level Duration: 26 weeks 	<ol style="list-style-type: none"> Body weight; 2) body mass index; 3) waist circumference; 4) body composition; 5) exercise capacity; 6) serum levels of hormones; 7) glucose tolerance; 8) reported side-effects; 9) psychological characteristics; 10) lipid profile; 11) blood counts; 12) bone markers; 13) liver enzymes
NCT 00241436	Non-randomized Phase II Completed	35 men with gynecomastia	<ol style="list-style-type: none"> Males aged 11–18 years; Gynecomastia, one breast measuring ≥2 cm in diameter; Normal renal liver and thyroid function; No evidence of hormone producing tumor and hypogonadism or androgen resistance 	Anastrozole, PO; Dose was not available Duration: 6 months	<ol style="list-style-type: none"> Anastrozole pharmacokinetics; Response rate

Continued

Table V Continued

Registration ID	Research type and status	Patients' condition and numbers	Inclusion criteria	Arms and interventions	Main outcome measures
NCT 02959853	RCT Phase IV Completed	23 severely obese veterans (pilot) with hypogonadotropic hypogonadism	<ol style="list-style-type: none"> Obese men (BMI ≥ 35) aged 35–65 years; Serum T < 300 ng/dl, LH < 9 U/l, E₂ > 40 pmol/l; Normal FT4, TSH, ACTH, IGF-I etc. 	<ol style="list-style-type: none"> Weight loss Anastrozole plus weight loss <p>Dose was not available Duration: 6 months</p>	<ol style="list-style-type: none"> Muscle strength; symptoms score of hypogonadism; fat mass; bone mineral density; visceral adipose tissue; bone quality
NCT 03933618	RCT Phase II Completed	24 men with erectile dysfunction and hypogonadal symptom	<ol style="list-style-type: none"> Men age 18–70 years, BMI < 40; Baseline morning T 150–350 ng/dl; LH 1.5–9.2 mIU/ml, FSH 1.6–8.0 mIU/ml, prolactin 4–15 ng/ml; Positive androgen deficiency in the aging male (ADAM) score; Sexual health inventory for men (SHIM) score > 7 and < 21; Men willing not to take phosphodiesterase 5 inhibitors 	<p>Anastrozole (AZ) 1 mg, clomiphene citrate (CC) 25 mg, and placebo, daily, each for 8 weeks in the following order:</p> <ol style="list-style-type: none"> AZ-CC-placebo AZ-placebo-CC CC-AZ-placebo CC-placebo-AZ Placebo-CC-AZ 6) Placebo-AZ-CC 	<ol style="list-style-type: none"> IIEF (international index of erectile function) Score (every 8 weeks); Serum T, FSH, LH, E₂ and SHBG levels; ADAM (androgen deficiency in the aging male) score; EHS (erectile hardness score)

PSA, prostate-specific antigen; DEXA, dual energy X-ray absorptiometry; ACTH, adrenocorticotrophic hormone; TSH, thyroid stimulating hormone; FT4, free thyroxine; IGF, insulin-like growth factor; RCT, randomized controlled trial.

Table VI CYP19A1 polymorphisms and clinical relevance.

Subjects	Polymorphisms	Results	Reference
100 gynecomastia male and 99 controls	SNP location: rs10046 at 3'-UTR; Variant: C/T	The TT genotype was significantly higher in patients. Individuals with the T allele showed higher risk of developing gynecomastia.	Czajka-Oraniec et al. (2008)
67 patients with breast cancer treated with letrozole	SNP location: rs4646 at 3'-UTR; Variant: T/G	Allele T was associated with improved treatment efficacy of letrozole and improved time to progression.	Colomer et al. (2008)
95 women with breast cancer treated with letrozole	SNP location: rs4646 at 3'-UTR; Variant: C/A	Variants AC/AA were associated with poor response to letrozole and lower progression-free survival than the CC genotype.	Garcia-Casado et al. (2010)
52 women with ER ⁺ breast cancer treated with AIs	SNP location: rs6493497 and rs7176005 at exon 1.1; Variant: C/T and G/A	The two SNPs resulted in greater inhibition of aromatase activity, and were also associated with higher plasma E ₂ levels pre- and post-AIs treatment.	Wang et al. (2010)
308 women with ER ⁺ breast cancer treated with AIs	SNP location: rs4775936 at exon 1.6; Variant: C/T	T allele was associated with improved therapeutic efficacy and time to treatment failure than the reference allele.	Ferraldeschi et al. (2012)
300 Caucasian men more than 55 years old	TTTAn in Intron 4	Men with a high-repeat genotype (>9 repeats) showed higher E ₂ , E ₂ :T and free estrogen index than men with a low-repeat genotype (<9 repeats).	Gennari et al. (2004)

Continued

Table VI Continued

Subjects	Polymorphisms	Results	Reference
31 obese Caucasian men who had gastric bypass surgery and 118 controls	TTTAn in Intron 4	The correlation between E ₂ and weight was seen only among men with higher TTTA repeats, who exhibited reduced E ₂ level after weight loss. Higher TTTA repeats were associated with a strengthened relationship between obesity and E ₂ .	Hammoud et al. (2010a)
196 men recruited from the general community with various BMI	TTTAn in Intron 4	Men with high repeats (>7 repeats) had higher E ₂ and lower FSH levels, who also presented a negative correlation between BMI and sperm count, and exhibited increased E ₂ and decreased T:E ₂ ratio with increasing weight.	Hammoud et al. (2010b)

UTR: untranslated region; ER: estrogen receptor; SNP, single nucleotide polymorphism; TTTAn, tetranucleotide TTTA repeat polymorphism.

Table VII Side-effects of aromatase inhibitors in treating male infertility.

Side-effects	Drug	Patients (n/N)	Total (n/N, %)	Results	Reference
Rash	Anastrozole	1/13	5/39, 12.8%	Dropped out	Helo et al. (2015)
	Letrozole	2/22		Mild, medication continued	Cavallini et al. (2013)
	Letrozole	2/4		Mild, medication continued	Cavallini et al. (2011)
Dry mouth	Anastrozole	1/86	3/110, 2.7%	Dropped out	Shoshany et al. (2017)
	Letrozole	2/24		Mild, medication continued	Shuling et al. (2019)
Weakness	Letrozole	2/15	5/39, 12.8%	Transient, medication continued	Gregoriou et al. (2012)
	Letrozole	3/24		Mild, medication continued	Shuling et al. (2019)
Fatigue	Letrozole	5/24	5/24, 20.8%	Mild, medication continued	Shuling et al. (2019)
Nausea	Letrozole	1/15	2/39, 5.1%	Mild, lasted for 10 days, medication continued	Gregoriou et al. (2012)
	Letrozole	1/24		Mild, medication continued	Shuling et al. (2019)
Stomach discomfort	Letrozole	1/24	1/24, 4.2%	Mild, medication continued	Shuling et al. (2019)
Headache	Letrozole	2/15	11/80, 13.8%	Mild, medication continued	Gregoriou et al. (2012)
	Letrozole	2/27		Mild, medication continued	Saylam et al. (2011)
	Letrozole	6/24		Mild, medication continued	Shuling et al. (2019)
	Anastrozole	1/14		Mild, medication continued	Gregoriou et al. (2012)

Continued

Table VII Continued

Side-effects	Drug	Patients (n/N)	Total (n/N, %)	Results	Reference
Loss of libido	Letrozole	5/22	23/136, 16.9%	Mild, medication continued	Cavallini et al. (2013)
	Letrozole	4/4		Mild, medication continued	Cavallini et al. (2011)
	Letrozole	13/24		Mild, medication continued	Shuling et al. (2019)
	Anastrozole	1/86		Dropped out	Shoshany et al. (2017)
Loss of hair	Letrozole	5/22	7/46, 15.2%	Mild, medication continued	Cavallini et al. (2013)
	Letrozole	2/24		Mild, medication continued	Shuling et al. (2019)
Weight gain	Letrozole	1/24	1/24, 4.2%	Mild, medication continued	Shuling et al. (2019)
Ocular symptoms	Anastrozole	1/86	1/86, 1.2%	Ocular pruritus turning into ocular pain, dropped out	Shoshany et al. (2017)
Increase of liver enzymes	Letrozole	1/15	3/29, 10.3%	Transient GOT and GPT increase, medication continued	Gregoriou et al. (2012)
	Anastrozole	2/14		Transient ALP increase, medication continued	Gregoriou et al. (2012)
Diarrhea	Anastrozole	1/14	3/45, 6.7%	Mild, lasted for 3 days, medication continued	Gregoriou et al. (2012)
	Anastrozole	2/31		Dropped out	Mechlin et al. (2014)
Constipation	Anastrozole	1/31	1/31, 3.2%	Dropped out	Mechlin et al. (2014)
Joint and tendon pain, and swollen limbs	Anastrozole	2/86	2/86, 2.3%	Dropped out	Shoshany et al. (2017)
Irritability, depression, or nervousness	Anastrozole	1/86	3/114, 2.6%	Dropped out	Shoshany et al. (2017)
	Letrozole	1/4		Mild, medication continued	Cavallini et al. (2011)
	Letrozole	1/24		Mild, medication continued	Shuling et al. (2019)
Breast tenderness	Anastrozole	1/86	1/86, 1.2%	Dropped out	Shoshany et al. (2017)
Nose bleed	Letrozole	1/24	1/24, 4.2%	Mild, medication continued	Shuling et al. (2019)
Frequent urination	Letrozole	1/24	1/24, 4.2%	Mild, medication continued	Shuling et al. (2019)
Bad sleep	Letrozole	1/24	1/24, 4.2%	Mild, medication continued	Shuling et al. (2019)

GOT, glutamic oxalacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase.

and decreased male fertility, which could also be ameliorated by AIs. Polymorphisms of the aromatase *CYP19A1* gene may be related to aberrant aromatase activity and affect the treatment efficacy of AIs, findings which still need to be validated by further basic and clinical

research. However, most evidence comes from non-randomized cohort studies and case studies, and RCTs are required to evaluate the efficacy and risks of using AIs in treating male infertility before a medication guideline can be made for clinical practice.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Authors' roles

L.Z. had the major role of conceiving the idea and leading the work. Y.C. and L.P. contributed in the writing, preparing the images, gathering and analyzing the reported data.

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Conflict of interest

There is no conflict of interest to declare.

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