The Efficacy and Safety of Letrozole or Anastrozole in The Treatment of Specific Male Infertility Patients: Meta-Analysis and Systematic Review

Bin Guo

Shaanxi University of Chinese Medicine

Jiao-jiao Li

Shaanxi University of Chinese Medicine

Ya-ling Ma

Shaanxi University of Chinese Medicine

Yu-tao Zhao

Shaanxi University of Chinese Medicine

Jian-guo Liu (✉ kukuliushui@126.com)

Shaanxi Provincial Hospital of traditional Chinese Medicine Xi'an 710000

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Abstract

Background: Letrozole (LE) or anastrozole (AZ) is clinically beneficial in male infertility patients with a low testosterone-estradiol ratio (T/E2). Many scholars believe it has the potential to become one of the effective drugs to treat male infertility. But some relevant research results are different or even the opposite.

Study Question: The purpose of this report is to evaluate the efficacy and safety of letrozole or anastrozole in the treatment of specific male infertility patients.

Data Sources: We performed a comprehensive literature search using PubMed, Embase, Cochrane, CNKI, VIP, CBM, and Wanfang Date through August 2021 for all studies.

Study Design: We conducted a systematic review with meta-analysis of the all available literature reporting sperm conventional parameters, gonadotropin and testosterone levels, and/or the pregnancy rate.

Results: The total of 10 studies involving 280 patients were included. LE or AZ administration increased significantly sperm concentration, total sperm count, and serum luteinizing hormone, follicle-stimulating hormone, testosterone levels and T / E2 compared with baseline values, but E2 levels were significantly reduced. In contrast, LE or AZ did not have any significant effect on sperm concentration and motility and pregnancy rate, but improved total sperm count, sperm morphology, compared to the control group, which included studies done with Selective estrogen receptor modulators (SEMR) or testosterone undecanoate (TU).

Conclusion: LE or AZ may be effective in the treatment of low T / E2 male infertility, perhaps better than other anti-estrogen or exogenous testosterone supplementation. In addition, we should pay special attention to the changes of E2 during treatment.

1. Introduction

In twenty-first century, infertility has been regarded as the third-ranking disease following cancer and cardio-vascular diseases¹. About 80 million couples of childbearing age worldwide face infertility, and are growing at a rate of 2 million a year. Male factors account for about 50%, of which 30 – 40% can not be explained as idiopathic male infertility². Testicular spermatogenesis dysfunction is considered to be an important reason for male infertility, which is often manifested as semen quality abnormalities such as oligospermia and asthenospermia³.

Treatments for male infertility are diverse, among which empirical drug therapy is considered to have the potential to improve semen quality, but it is still widely controversial⁴. For example, selective estrogen receptor modulators (SERM), represented by clomiphene (CC) and tamoxifen (TAM), are among the most popular and recognized therapies, while their efficacy is not universally accepted and their side effects are worrisome. Reseaches have confirmed that TAM treatment of male infertility increasees the sperm count, but the effect on sperm motility and morphology has not been observed^{5,6}. The latest meta-analysis⁷ suggested that TAM only improved sperm count and morphology, and had no significant effect on sperm motility and pregnancy rate. In addition, it has been reported in the study that patients with oligospermia developed into azoospermia after CC treatment⁸. Therefore, it is very important to explore effective treatment methods in line with scientific concepts.

The third generation of Aromatase inhibitors (AIs) —Letrozole (LE) and Anastrozole (AZ), as new anti-estrogen therapy drugs, was initially the first-line treatment of estrogen receptor-positive breast cancer. With the in-depth study of AIs, scholars have found⁹ that AIs are benefit for specific infertility groups, namely male infertility patients with T / E2 < 10. The Meta-data from three years ago showed¹⁰ that AIs promote the semen quality of these specific groups, while also

greatly improving the T / E2 ratio, but the pregnancy rate was not reported. Recently, some scholars believe¹¹ that Als treatment of male infertility should not be limited to the reference of T / E2 < 10, and also effective in normal T / E2 for male infertility.

Therefore, in order to systematically and comprehensively understand the efficacy and cutting-edge dynamics of Als in treating male infertility, we have conducted a meta-analysis and systematic review of published clinical trials. Finally, it is expected to provide the latest evidence for the efficacy of Als in the treatment of male infertility.

2. Materials And Methods

2.1 Registered

The protocol for this systematic review was registered on INPLASY (INPLASY202190062) and is available in full on the inplasy.com

2.2 Search strategy

Two independent researchers searched PubMed, EMBASE, Cochrane, CNKI, VIP, CBM and WanFang Date respectively. Publication dates are limited to August 2021 from the establishment of the database. Keywords and phrases, including medical subject words (MeSH) related to this study and other related terms are used to search. Taking PubMed as an example, the search strategy is as follows: ('Anastrozole' OR 'Aromatase inhibitor' OR 'Letrozole') and ('Male infertility' OR 'Asthenozoospermia' OR 'Oligospermia' OR ' Oligoasthenoteratozoospermia' OR ' Male subfertility' OR 'Male factor infertility' OR 'Sperm Parameters')

2.3 Inclusion and exclusion criteria

Data were collected on study design, year of publication, country, and mean age of patients. The following criteria were included in the meta-analysis study:

Inclusion criteria include:

- (1) Subjects: clinically diagnosed as a non-azospermia patients with male infertility, and T/E2 < 10. (T/E2, T: ng / dl; E2: pg / ml)
- (2) Intervention methods: randomized controlled and uncontrolled clinical trials.
- (3) Intervention measures: use LE or AZ to intervene.
- (4) Observation indicators: (i). Main outcome indicators: total sperm count, sperm concentration, sperm motility, sperm morphology, total functional sperm fraction (TFSF). (ii). Secondary outcome measures: serum follicle stimulating hormone (FSH), luteinizing hormone (LH), mate pregnancy rate, estrogen (E2), testosterone (T), and testosterone to estradiol ratio (T / E2).

Total functional sperm fraction (TFSF): total functional sperm fraction is a comprehensive indicator of semen quantity and quality. The formula used is: total sperm count × percentage of rapid linear progressive movement × percentage of normal morphology × 10⁻⁴. This value can well and comprehensively reflect the normal fraction of sperm in terms of morphology and motility during ejaculation.

Exclusion criteria include:

(1) Decreased male semen quality due to known causes (male parainglandular infection, varicocele, Y chromosome microdeletion, Klinefelter syndrome, testicular torsion or trauma, history of cryptorchidism)

(2) Patients with ejaculation disorder.

(3) Studies on serious loss of original data.

The selection criteria for included studies (population, intervention, comparison, and outcome [PICO]) are shown in Table 1. Studies that did not meet these criteria were excluded from the analysis.

Table 1
Criteria in included studies (PICO)

included		Excluded
Population	The clinical diagnosis was non- aspermic patients with male infertility, and T / E2 < 10..	(1) Other factors lead to decreased semen quality in men (male accessory gland infection, varicocele, Y chromosome microdeletion, Klinefelter syndrome, testicular torsion or trauma, history of cryptorchidism) (2) Patients with ejaculation disorder.. (3) Studies on the serious loss of original data.
Intervention	Intervention with letrozole or anastrozole	
Comparison	Semen analysis performed at baseline was compared with that performed after the intervention. The intervention result of Letrozole /Anastrozole compared to the intervention result of SERM/TU control group.	
Outcomes	The main: Sperm concentration, Sperm total count, Sperm motility, Sperm morphology Secondary: luteinizing hormone, follicle- stimulating hormone, testosterone, functional sperm fraction, and pregnancy rate	
Study type	Controlled and not-controlled clinical trial, Retrospective study	

2.4 Quality assessment of relevant studies

First, we will use the Ottawa-Newcastle scale (ONS) to assess the quality of all studies as shown in Table 2, involving the selection of study groups, comparability between groups, and determination of prognosis ¹². The full score is 9. Studies with a score <5 were considered to have a high risk of bias, between 5 and 7 moderate risk, and >7 low risk of bias.

Table 2
The main characteristics of the included literatures and their quality evaluation

Source	Year	Study design	Participants	Intervention	Trial duration	Outcome	NOS
Saylam et al.	2011	Prospective clinical trial	Total: 27 ● azoospermia (n = 17) ● oligoasthenospermia(n = 10)	● Letrozole 2.5 mg daily	6 months	Sperm count, Sperm motility, sperm concentration, FSH, LH, T, E2, T/E2	7
Gregoriou et al.	2012	Prospective randomized controlled clinical trial	Total: 29	● Letrozole 2.5 mg daily (n = 15) ● Anastrozole 1 mg daily (n = 14)	6 months	Sperm count, Sperm motility, sperm concentration, FSH, LH, T, E2, T/E2, TFSF	8
Shoshany et al.	2017	Retrospective study	Total: 86 ● oligozoospermia(n = 21) ● Other(n = 65)	● Anastrozole 1 mg daily	4 months	Sperm count, Sperm motility, sperm concentration	7
Helo et al.	2015	Prospective, randomized, double-blind	Total: 26	● Clomiphene citrate 25 mg daily (n = 13) ● Anastrozole 1 mg daily (n=13)	3 months	Sperm motility, sperm concentration FSH, LH, T, E2, T/E2	8
Başar et al.	2009	Prospective clinical trial	Total: 32	● Anastrozole 1 mg daily (n=32)	2 months	Sperm count, Sperm motility, sperm morphology, FSH, LH, T, E2, T/E2	6

Abbreviations: LH: luteinizing hormone; FSH: follicle-stimulating hormone; T: testosterone; NOS: Newcastle-Ottawa Scale.

* TFSF: TFSF was estimated by multiplying total sperm count ($\times 10^6$) by motility (%) and by morphology (%);

Source	Year	Study design	Participants	Intervention	Trial duration	Outcome	NOS
Li xiaoxia et al.	2019	Randomized controlled clinical trial	Total: 150	<ul style="list-style-type: none"> ● Tamoxifen 20 mg a day (n = 50) ● Letrozole 2.5 mg daily (n = 50) ● Placebo tablets (n = 50) 	3 months	Sperm concentration, morphology, Sperm count ,FSH, LH, T, E2, T/E2, TFSF	8
Source	Year	Study design	Participants	Intervention	Trial duration	Outcome	NOS
Tian et al.	2016	Randomized controlled clinical trial	Total: 100	<ul style="list-style-type: none"> ● Letrozole 2.5 mg daily (n = 50) ● T undecanoate 80 mg a day (n = 50) 	3 months	Sperm count, Sperm motility, sperm concentration, sperm morphology, FSH, LH, T, E2, T/E2, TFSF, Pregnancy rate	7
Peivandi et al.	2019	Prospective clinical trial	Total: 41	<ul style="list-style-type: none"> ● Letrozole 2.5 mg daily (n = 41) 	4 months	Sperm motility, sperm concentration, sperm morphology, FSH, LH, T, E2, T/E2	7
Tian Fuying et al.	2016	Randomized controlled clinical trial	Total: 155	<ul style="list-style-type: none"> ● Letrozole 2.5 mg daily (n = 14) ● T undecanoate 40 mg a day (n = 16) ● HCG 2000U two times a week (n = 65) ● Zn, VitE and WuZiYanZong pill (n = 60) 	3 months	Sperm count, Sperm motility, sperm concentration, sperm morphology, FSH, LH, T, E2, T/E2, TFSF	6

Abbreviations: LH: luteinizing hormone; FSH: follicle-stimulating hormone; T: testosterone; NOS: Newcastle-Ottawa Scale.

* TFSF: TFSF was estimated by multiplying total sperm count ($\times 10^6$) by motility (%) and by morphology (%);

Source	Year	Study design	Participants	Intervention	Trial duration	Outcome	NOS
Kooshesh et al.	2020	Retrospective study	Total: 20	●Letrozole 2.5 mg daily (n = 14)	3 months	Sperm motility, sperm concentration, sperm morphology, FSH, LH, T, E2, T/E2, Pregnancy rate	7
Abbreviations: LH: luteinizing hormone; FSH: follicle-stimulating hormone; T: testosterone; NOS: Newcastle-Ottawa Scale.							
* TFSF: TFSF was estimated by multiplying total sperm count ($\times 10^6$) by motility (%) and by morphology (%);							

Subsequently, the Cochrane bias risk tool was used to evaluate the study quality of the RCTs¹³ as shown in Table 3. This assessment was based on six criteria:

Table 3
Risk of bias assessment of included studies.

Source/Year	A	B	C	D	E	F	G
Tian et al. 2016	?	?	□	□	□	□	□
Li xiaoxia et al. 2019	+	?	□	□	□	-	□
Helo et al. 2015	+	?	□	□	□	□	□
Tian Fuying et al. 2016	?	?	□	□	-	□	-
Evaluation criteria: A. Random sequence generation ; B. Allocation concealment; C. Blinding of participants; D .Blinding of outcome assessment; E. Incomplete outcome data; F. Selective reporting; G. Other bias							
+: low risk; -: high risk; ?: unclear							
Figure:							

(1)How did the author generate the random sequences (selection bias)?

(2) Was the allocation be hidden (selection bias)?

(3)Did the subjects, researchers and reviewers know the distribution and intervention measures (monitoring bias)?

(4) Did the author explain lost data (loss bias)?

(5) Did the author selectively report the results (reporting bias)?

(6) Was there any other possible bias (other bias)?

The risk of bias was assessed as “low”, “high” or “unclear”. A quality score > 3 points (4-7points) indicated a high-quality study.

Our independent researchers screen studies against inclusion and exclusion criteria, cross-check the screening results and generate tables to collect data. If there is a disagreement, consult a third party before making a decision.

2.5 Data processing and statistical data analysis

In data collection, we find that different research articles report some summary statistics in different forms. For continuous random variables, the most typical aggregate statistics are sample mean and sample standard deviation ($M \pm SD$). For some reasons, some studies reported median, first and third quartiles (M, q_{25}, q_{75}) and / or maximum and minimum values. This leads to inconsistent aggregate statistics provided by different empirical studies, making it impossible to perform effective meta-analysis. If only studies that report sample mean and sample standard deviation are retained. This obviously lost a lot of useful information.

Therefore, how to properly convert median, extreme or quartile to mean and standard deviation is very important. We use the online calculator compiled by Professor Tong Tie jun of Mathematics Department of Hong Kong Baptist University for data conversion. The conversion logic of the computer is based on the statistical research of J. Shi et al^{14,15}, D. Luo et al¹⁶ and X. Wan et al¹⁷. The specific links are as follows:

<http://www.math.hkbu.edu.hk/~tongt/papers/median2mean.html>

Meta-analysis was performed using RevMan 5.4 software. First, heterogeneity tests were carried out among studies. If there was no heterogeneity ($I^2 < 50\%$, $P > 0.1$), the fixed effect model was used for analysis; otherwise, the random effect model was used for analysis¹⁸. For each outcome, the mean difference (MD) or the standard mean difference (SMD) with the 95% confidential interval (CI) was calculated. Sensitivity analysis and publication bias detection were conducted for the indicators with a large number of included studies.

3. Results

3.1 The search results

A total of 319 items were retrieved using the above retrieval strategy. After excluding duplicate records (86 articles), 233 articles were screened. Of these, 194 were deemed irrelevant for research purposes, and the remaining 39 full texts will be evaluated for eligibility. The PICO of 25 studies that did not conform to Table 1 were excluded, and four studies were excluded due to unavailable experimental data. Finally, 10 articles^{19–28} met the inclusion criteria for inclusion analysis (Figure 1), including 3 articles in Chinese^{19–21} and 7 articles in English^{22–28}.

3.2 Basic information included in the study

Ten articles were included, including a total of 280 adult male patients. All the included studies reported the general information of the subjects completely, and there was no significant difference in the basic information of patients such as age and disease course. Baseline comparison and duration of treatment were described in all literature, with a minimum of 2 months and a maximum of 6 months.

Among the research objects, research objects of Saylam et al²⁷ were 17 azoospermia patients and 10 oligoaspermia patients. Study of Shoshany et al²⁸ included 65 cases of male infertility, 21 of which were oligoaspermia. Data on the above subjects are available. In data reports, most of them adopt the mode of mean and standard deviation, except that Li et al²¹ and Tian et al²⁰ adopt the form of interquartile rang when reporting semen concentration and total sperm count. Adverse reactions were reported in six studies^{20,21,23–25,28}. Two studies^{19,25} reported pregnancy rates. The basic information of each included study is shown in Table 2.

3.3 Main Research Indicators

3.3.1 Sperm concentration.

Compared with the baseline, Als significantly increase sperm concentration (SMD: 2.25; 95% CI: 1.25 to 3.25; $P < 0.001$) (Figure 2). Subgroup analysis showed that severe oligozoospermia (SMD: 3.06; 95% CI: 1.98 to 4.15; $P < 0.001$), moderate oligozoospermia (SMD: 2.68; 95% CI: 0.86 to 4.51; $P < 0.001$) and mild oligozoospermia (SMD: 2.04; 95% CI: 1.53 to 2.55; $P < 0.001$) were significantly improved, but not including normal concentrations (SMD: -0.52; 95% CI: -1.30 to 0.27; $P < 0.001$). This difference may be caused by the single literature included by the latter. There is heterogeneity among studies ($P < 0.05$, $I^2=95\%$). We conduct sensitivity analysis and adopt the method of eliminating one by one to show that the research results are robust, but heterogeneity sources has not been found. The analysis from controlled studies showed that the increase of sperm concentration reach the statistical significance compared with the control group made up of patients receiving SREM or TU (SMD: 1.27; 95% CI: -0.33 to 2.87; $P = 0.12$) (Figure 3). studies in Tian et al²⁰ and Helo et al²⁴ were considered sources of heterogeneity ($P < 0.05$, $I^2=96\%$). Excluding these two articles, heterogeneity disappeared ($P = 0.24$, $I^2=26\%$), but with statistical significance (SMD: 1.47; 95% CI: 0.98 to 1.95; $P < 0.05$), suggesting that the results are not robust.

3.3.2 Total sperm count.

Total sperm count also improved significantly after Als treatment (WMD: 6.6; 95% CI: 2.54 to 10.66; $P = 0.001$), and heterogeneity was found between studies ($P < 0.05$, $I^2=98\%$) (Figure 4). After sensitivity analysis, heterogeneity sources has not been found, but the results were robust and statistically significant. Compared with the control group, the total sperm count improved significantly (WMD: 9.96; 95% CI: 6.23 to 13.66; $P < 0.05$), but there were also high heterogeneity ($P = 0.0001$, $I^2=89\%$) (Figure 5). Tian's research²⁰ is the source of heterogeneity, and the heterogeneity disappears after elimination ($P < 0.05$, $I^2=0\%$), and there are still significant differences (WMD: 8.11; 95% CI: 6.39 to 9.86; $P < 0.05$).

3.3.3 Sperm motility.

Compared with the baseline value, sperm motility was significantly ameliorate after Als treatment (WMD: 8.54; 95% CI: 3.42 to 13.66; $P = 0.001$), and heterogeneity was found in the study. ($P < 0.05$, $I^2=98\%$) (Figure 6). We used the method of eliminating one by one for sensitivity analysis, and the results were robust and always statistically significant. Compared with the control group, Als had no significant advantage in improving sperm motility (WMD: 5.36; 95% CI: -5.59 to 16.32; $P = 0.34$) (Figure 7), and the results were robust.

3.3.3 Sperm morphology.

Sperm morphology ameliorate compared with baseline (WMD: 2.48; 95% CI: 1.92 to 3.05; $P < 0.05$) (Figure 8). Basar et al²², Li et al²¹ and Tian et al²⁰ were the sources of heterogeneity ($P < 0.05$, $I^2=92\%$). Delete these three literature heterogeneity disappear ($P = 0.57$, $I^2=0\%$), and the statistical difference remains unchanged (WMD: 2.98; 95% CI: 2.70 to 3.26; $P < 0.05$). Als were also significantly different from the control group (WMD: 1.51; 95% CI: 0.65 to 2.37; $P = 0.0006$) (Figure 9).

3.4 Secondary research indicators

3.4.1 Total functional sperm fraction.

The functional sperm fraction was used as a comprehensive index of sperm quality. It was estimated using the formula total number $\times$ percentage of rapid and linear progressive motility $\times$ percentage of normal forms $\times 10^{-4}$. This value objectively and comprehensively reflects the quality of semen during one ejaculation. The functional sperm fraction also significantly improved compared with before treatment (WMD: 0.95; 95% CI: 0.36 to 1.57; $P = 0.002$), but there was high heterogeneity ($P = 0.002$, $I^2=80\%$) (Figure 10). The source of heterogeneity is Tian et al²⁰, ignoring the heterogeneity disappeared ($P = 0.86$, $I^2=0\%$), still has statistical significance (WMD: 0.70; 95% CI: 0.38 to 1.02; $P < 0.05$). Compared with the control, Als significantly increased the functional sperm fraction (WMD: 1.05; 95% CI: 0.14 to 1.96; $P = 0.02$) (Figure 11).

3.4.1 Sex hormones.

Compared with before treatment, Als significantly increased serum FSH (SMD: 0.97; 95% CI: 0.02 to 1.92; P = 0.04) (Figure 12) and LH levels (SMD:0.87; 95% CI:0.13 to 1.60; P = 0.02) (Figure 13). Sensitivity analysis showed that FSH results were not robust, and the results were not consistent in different literatures. At the same time, Als treatment significantly increased baseline T level (SMD: 3.58; 95% CI: 2.33 to 4.82; P = 0.04) (Figure 14) and decreased E2 level (SMD:-3.58; 95% CI:-5.13 to -2.03; P = 0.04) (Figure 15). T/E2 ratio also increased significantly (SMD: 4.71; 95% CI: 3.69 to 6.92; P<0.05) (Figure 16).

3.4.1 Pregnancy rate.

Kooshesh et al²⁵ and Tian Fuying et al¹⁹ reported pregnancy rates. In the study of Tian Fuying et al¹⁹, the LE group reported 1 pregnancy(1/14), which had no advantage over another control groups, including TU group (0 / 16), HCG group (11 / 65) and traditional Chinese medicine group (5 / 60)(OR:0.76; 95% CI:0.21 to 2.70; P = 0.67) (Figure 17). Kooshesh et al study reported 4 pregnancies (4 /20).

3.5 Drug side effects

Side effects were reported in six articles^{20,21, 23–25,28}. The reported side effects were not life-threatening and resolved after treatment was suspended or continued. Of 362 patients who received anastrozole and letrozole, 29 (8%) had side effects. Among the different adverse events, the most representative was decreased/lost libido, followed by subclinical liver dysfunction, skin rash and another, with 4.1% (15/362), 1.38% (5/362), 0.9%(3/362) and 0.27%(1/362) respectively. All two drugs administered were associated with episodes of subclinical liver dysfunction, described as asymptomatic elevated liver function tests and/or mildly elevated transaminase and bilirubin values. Studies also reported side effects of joint muscle pain, nausea, and reports of fatigue.

4. Discussion

4. DISCUSSION

Aromatase inhibitors were first used to treat oligozoospermia in 1981 and showed benefits²⁹. Subsequently, Pavlovich⁹ proposed 10 as the lower limit of the normal value of testosterone - estradiol ratio based on observation in 2001. Since then, more and more studies have shown that aromatase inhibitors play a positive role in the treatment of male infertility with reduced T/E2. Currently, clinically commonly used Als includes testosterone, letrozole, and anastrozole, but testosterone is no longer used clinically in the United States due to its unsatisfactory efficacy in well-designed experiments, as well as its hormone-like effects and risk of inhibiting adrenocorticosteroids^{30,31}.

Therefore, this study conducted a meta-analysis of the safety and efficacy of letrozole or anastrozole in the treatment of male infertility with T/E2 < 10 without asspermatozosis. The results showed that the semen parameters (sperm concentration, sperm total, sperm motility) and hormone parameters (FSH, LH, T) of patients treated with LE or AZ were significantly increased, while E2 was significantly decreased compared with the pretreatment values. This may be related to Als inhibition of aromatase activity widely existing in testicular germ cells, brain, fat and other tissues, which blocks the transformation of testosterone to estradiol, resulting in increased endogenous testosterone and decreased estrogen.

As well kown, Androgens and estrogen play an important role in male spermatogenesis. Spermatogenesis highly depends on the high concentration of androgen (T) in testis, and the reduction of androgen may lead to spermatogenesis defect³². On the contrary, high serum E2 has a strong negative feedback inhibitory effect on hypothalamic-pituitary axis, which can inhibit FSH and LH and hinder spermatogenesis. Therefore, too high E2 concentration and/or too low androgen concentration (low T/E2) will damage the spermatogenic function of testis, resulting in spermatogenic

dysfunction, and ultimately male infertility³³. Als absolve the negative feedback inhibition of estrogen on HPG axis by reducing estrogen, thereby promoting the release of FSH and LH from pituitary gland. FSH acts on spermatogenic tubules of testis and promotes spermatogenic cell proliferation and division. LH acts on testicular interstitial cells to promote testosterone secretion. Two hormones synergistically promote spermatogenesis. In addition, Als can increase the concentration of androgen in the testis. Peter N³⁰ believes that most aromatase activity comes from testicles rather than external turnover, because obese people with more fat do not benefit more from it.

Side effects were also reported during treatment with LE or AZ, but were not life-threatening and were resolved after treatment was discontinued. Among the adverse events, the most representative is decreased libido. This may be because estradiol increase libido in men with low testosterone levels. Conversely, in men with normal testosterone levels, sexual desire decreased after estradiol treatment. Thus, the use of LE or AZ in men with low testosterone levels appears to reduce libido by lowering estradiol³⁴⁻³⁶. In general, letrozole and anastrozole have few side effects and are well tolerated.

However, it is worth mentioning that since the release of Pavlovich research⁹, people have paid more attention to Als in the treatment of male infertility patients with $T / E2 < 10$, while ignoring other possibilities. The latest report in 2019¹¹ innovatively extended the application of Als treatment to patients with normal T/E2 ratio. Fifteen patients with severe oligozoospermia (sperm concentration $< 5 \times 10^6 / \text{mL}$) with T/E2 ratio > 10 were treated with LE 2.5 mg daily for 4 months. After treatment, mean sperm concentration increased 5.5 times, serum T concentration significantly increased, E2 concentration significantly decreased, and T/E2 ratio significantly increased ($P < 0.0001$). But the sample size of this study was relatively small, and additional studies are needed to validate the findings.

And the most important thing. When using Als to treat male infertility, semen parameters should not only be used as the evaluation criteria for efficacy, but also changes in E2 levels and T/E2, which may represent aromatic enzyme activity in the body, should also be included in the evaluation, because it may affect sperm function and ultimately pregnancy. Study showed that round sperm cells of aromatase knockout mice developed acrosomal dysplasia in early spermatogenesis and decreased ICSI fertilization capacity by 50%^{37,38}. Lambard et al³⁹ found that the mRNA level of P450arom was lower in non-motile sperm than that in motile sperm in the same sample, suggesting that aromatase may be related to the acquisition of motility. It has also been observed that testosterone, as a substrate for aromatization, increase the motility or mobility of ejaculated sperm, and induce energy acquisition and acrosome reaction. These effects were inhibited by letrozole, a specific inhibitor. It seems that there is a linear relationship between the local estrogen produced by sperm and sperm capacitation and acrosome reaction, and the synthesis of estrogen in sperm may affect its fertilization ability⁴⁰. Arroyo MAM's research⁴¹ also has similar views. Their team found through rat experiments that long-term use of LE could lead to the reduction of estrogen, resulting in changes in reproductive characteristics and ultimately infertility. According to the study data of Helo et al²³, we were found that in male infertility patients with normal semen quality and hypogonadism (Normal E2 level), the decrease of E2 level was accompanied by the decrease of semen quality after oral AZ treatment. This seems to indicate that the impact of E2 on male reproduction is very subtle. What are the boundaries of E2 affecting male reproduction? This deserves further research and exploration.

Finally, there are some limitations in this study. Differences in study design, treatment measures, treatment cycle, outcome indicators and other aspects represent the bias of these studies, which is the source of heterogeneity. In addition, the data reported in the study with interquartile rang is still a bias despite reasonable and scientific conversion. So we can only be careful to conclude that Als may be effective in the treatment of low T / E2 male infertility, perhaps better than other anti-estrogen or exogenous testosterone supplementation. However, this conclusion should be based on the focus on the dynamic changes of estrogen, because estrogen is very subtle for male reproduction. In all, the clinical

efficacy and safety of AIs in the treatment of male infertility still need to design scientifically rigorous multi-center, large-sample randomized double-blind controlled trials, while strict implementation, follow-up investigation and standardized reports are also extremely important to improve the quality of literature.

Abbreviations

LE
letrozole
AZ
anastrozole
AIs
Aromatase inhibitors
T/E2
testosterone-estradiol ratio
CC
clomiphene
TAM
tamoxifen
SEMR
selective estrogen receptor modulators
TU
testosterone undecanoate
LH
luteinizing hormone
FSH
follicle-stimulating hormone
T
testosterone
E2
estradiol
TFSF
Total functional sperm fraction

Declarations

Author contributions

JGL contributed ideas and designs, and critically modified this article. JJL and BG collected, studied and evaluated the literature. YLM extract data from each article, and YTZ draws tables and charts for review. JJL and BG drafted the paper together. All authors read and approved the final manuscript.

Competing interests

All authors declare no competing interests.

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Figures

Figure 1

Flowchart of the studies included in the meta-analysis.

Figure 2

LE or AZ administration in comparison with baseline values. (Sperm concentration)

Figure 3

LE or AZ group versus Control group. (Sperm concentration)

Figure 4

LE or AZ administration in comparison with baseline values. (Total sperm count)

Figure 5

LE or AZ group versus Control group. (Total sperm count)

Figure 6

LE or AZ administration in comparison with baseline values. (Sperm motility)

Figure 7

LE or AZ group versus Control group. (Sperm motility)

Figure 8

LE or AZ administration in comparison with baseline values. (Sperm morphology)

Figure 9

LE or AZ group versus Control group. (Sperm morphology)

Figure 10

LE or AZ administration in comparison with baseline values. (Functional sperm fraction)

Figure 11

LE or AZ group versus Control group. (Functional sperm fraction)

Figure 12

FSH compared to baseline. (Sex hormones)

Figure 13

LH compared to baseline. (Sex hormones)

Figure 14

T compared to baseline. (Sex hormones)

Figure 15

E2 compared to baseline. (Sex hormones)

Figure 16

T/E2 compared to baseline. (Sex hormones)

Figure 17

LE or AZ group versus Control group. (pregnancy rate)