Outcomes of anastrozole in oligozoospermic hypoandrogenic subfertile men

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Objective: To determine whether the change in sperm parameters in subfertile hypoandrogenic men treated with anastrozole is correlated to the magnitude of increase in testosterone (T) to estrogen ratio in men responding to treatment.
Design: Retrospective study.
Setting: Male fertility clinic.
Patient(s): The study group consisted of 86 subfertile hypoandrogenic men with low T/estradiol (E_2) ratio (n = 78) or a prior aversive reaction to clomiphene citrate (n = 8).
Intervention(s): All patients were treated with 1 mg anastrozole daily, administered orally.
Main Outcome Measure(s): Hormone analysis and semen analysis before and after treatment were performed. Hormone analysis included measurements of total T, E_2, sex-hormone binding globulin, albumin, FSH, and LH, and bioavailable T was calculated. Total motile sperm count was calculated from the semen analysis.
Result(s): In all, 95.3% of patients had an increased serum T and decreased serum E_2 after treatment with anastrozole. Sperm concentration and total motile counts improved in 18 of 21 subfertile hypoandrogenic oligozoospermic men treated with anastrozole. In these men the magnitude of total motile count increase was significantly correlated with the change in the T/E_2 ratio. No improvement was seen in semen parameters of men with azoospermia, cryptozoospermia, or normozoospermia at presentation.
Conclusion(s): Approximately 95% of men with hypoandrogenism responded with improved endocrine parameters, and a subset of oligozoospermic men (approximately 25% of all patients) displayed significantly improved sperm parameters. In that subset, increase in sperm parameters was correlated with the change in the T/E_2 ratio, which argues for a physiologic effect of treatment. (Fertil Steril® 2017;107:589–94. ©2016 by American Society for Reproductive Medicine.)
Key Words: Anastrozole, aromatase inhibitors, male infertility, oligozoospermia, testosterone to estradiol ratio

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Infertility affects 15% of all couples trying to conceive, and a male factor is involved in approximately 50% of these couples (1). Overall, 20% of all men undergoing an infertility evaluation will be diagnosed with an endocrine abnormality (1). The prevalence of hypoandrogenism in men with oligozoospermia, as defined by the World Health Organization (version 4), has been observed to be approximately 43% (2). This is similar to the prevalence of 45% in men with azoospermia due to spermatogenic dysfunction. In men with obstructive azoospermia, however, hypoandrogenism has a much lower incidence of approximately 16.7%, similar to that in the general population (2). Spermatogenesis is highly dependent on intratesticular testosterone (T) synthesis, and hypoandrogenism may result in a defective spermatogenesis (3). Nonetheless, hypoandrogenism is imperfectly correlated with semen parameters (4), and delineation of the subgroup of patients that would benefit from correction of hormonal abnormalities is needed.

Estrogen (E) has a role in male fertility, although it is incompletely understood. It is known to be important for normal spermatogenesis (5, 6), but high concentrations of E, perhaps combined with low concentrations of T, may be detrimental to spermatogenesis (7). Estrogen is produced by the enzyme aromatase, which converts T into estradiol (E_2) and androstenedione into estrone. Aromatase is a cytochrome P450 enzyme that is present in both the testicular germ cells and somatic

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cells, as well as in other tissues and organs, such as adipose tissue, liver, and brain (8, 9).

Selective E receptor modulators and aromatase inhibitors are frequently used as treatment in hypoandrogenic men desiring fertility. Anastrozole has been demonstrated to increase T concentrations and the T/E ratio (9). A small but growing number of studies support the efficacy of aromatase inhibition in the treatment of hypoandrogenic subfertile men (10–14). We aimed to assess the efficacy of anastrozole use in hypoandrogenic men by investigating whether a correlation exists between semen analysis changes and hormonal changes during treatment.

MATERIALS AND METHODS

Institutional review board approval was obtained. Hypoandrogenic subfertile men treated with anastrozole (1 mg daily) between January 2010 and May 2016 were included in this study. All patients were seen by one treating physician (C.N.) at a single male infertility center. Indications for anastrozole treatment included a low calculated bioavailable T (<155 ng/dL) and either a T (in ng/dL) to E (in pg/mL) ratio of <10 (n = 78) or a history of prior adverse reaction to clomiphene citrate (CC) (n = 8). Exclusion criteria were men with a history of sex chromosome disorder, past exogenous T use, or other concomitant hormonal treatment.

Data on demographics, medical history, physical examination, testicular longitudinal axis, side effects and adverse events, laboratory values, and semen analyses before and after treatment were collected. Hormone concentrations were assessed before treatment and at 3 weeks and 4 months after prescribing anastrozole. Hormonal analysis was obtained between 7:00 and 11:00 AM by venipuncture. Assays of sex hormone–binding globulin, E, albumin, FSH, LH, and total T were obtained at the University of Illinois at Chicago laboratory. Testosterone was measured by the quantitative electrochemiluminescent immunoassay. Estradiol was measured by quantitative high-performance liquid chromatography–tandem mass spectrometry. Bioavailable T was calculated according to the Vermeulen formula (15). Semen analyses were performed before treatment and at 4 months after treatment. Semen samples were collected by masturbation after 2 to 3 days of sexual abstinence and processed within 1 hour of ejaculation. All patients had two or more recent semen analyses before treatment, no longer than 6 months before treatment commencement. The semen analysis with the greatest total motile count was selected to represent pretreatment status. At least two centrifuged semen analyses were carefully examined before the diagnosis of azoospermia and cryptozoospermia. Side effects were recorded, and anastrozole was discontinued in cases of significant adverse events. Microsurgical testicular sperm extraction outcomes after treatment were also recorded.

For statistical analysis, variables are presented as median and interquartile range or mean ± SE, as appropriate. A matched-pairs analysis estimated significance of change in laboratory values and semen analyses with treatment. A subgroup analysis was performed of men with baseline oligozoospermia. In this group we used linear regression to identify correlations between changes in hormone concentrations and increase in semen parameters. We then used multiple regression analysis to control for age and FSH as possible confounders. Statistical tests were two-sided and were considered statistically significant when $P<.05$. Analyses were performed with IBM SPSS statistics, version 20.

RESULTS

A total of 86 men with a median age of 37 (32–41) years were included. The median duration of unprotected intercourse was 24 (18–48) months. In all, 28 patients (32.6%) were azoospermic, and 8 (9.3%) had cryptozoospermia. A history of cryptorchidism was reported by 11 patients. Four patients had a history of varicocelectomy (three oligozoospermic patients and one normozoospermic patient). Four patients had a non–clinically significant grade 1 varicocele at presentation, as they were associated with either normozoospermia or azoospermia. Mean testis longitudinal axis was 4.32 ± 0.09 cm bilaterally.

Three weeks after anastrozole was prescribed, T and bioavailable T increased from 258.4 ± 10.8 ng/dL and 128.8 ± 4.7 ng/dL to 509.2 ± 20.4 ng/dL and 297.5 ± 12.7 ng/dL, respectively (both $P<.0001$; Table 1). Estradiol concentrations decreased from 40.8 ± 1.9 pg/mL to 24.6 ± 2.1 pg/mL after 3 weeks of anastrozole treatment ($P<.0001$). The T/E_2 ratio significantly increased from 6.98 ± 0.33 to 34.5 ± 6.5 (Table 1). Over the 4 months of treatment, LH increased from 6.41 ± 0.89 IU/L to 10.7 ± 1.1 IU/L ($P<.0001$), and

TABLE 1

Hormonal analysis in 86 men with hypoandrogenism treated with anastrozole.

Parameter	Baseline	At 3 wk	At 4 mo	*P* value[a]
Total T (ng/dL)	258.4 ± 10.8	509.2 ± 20.4	449.9 ± 19.5	<.0001
Bioavailable T (ng/dL)	128.8 ± 4.7	297.5 ± 12.7	N/A	<.0001
E_2 (pg/mL)	40.8 ± 1.9	24.6 ± 2.1	23.2 ± 2.2	<.0001
T/E_2 ratio	6.98 ± 0.33	34.5 ± 6.5	24.2 ± 3	<.0001
SHBG (nmol/L)	25.6 ± 1.1	24.9 ± 1.2	N/A	NS
LH (IU/L)	6.41 ± 0.89	10.7 ± 1.1	N/A	<.0001
FSH (IU/L)	12.4 ± 2	19.4 ± 2.3	N/A	<.0001

Note: Values are mean ± SE. SHBG = sex hormone–binding globulin.
[a] Comparing baseline vs. 3-wk results.

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FSH increased from 12.4 ± 2 IU/L to 19.4 ± 2.3 IU/L ($P<.0001$). Comparing the means at 3 weeks and 4 months of treatment, these hormonal effects were maintained at 4 months of therapy ($P>.05$ for all). Four patients (4.7%) did not demonstrate an increase in T and decrease in E_2. Of the patients who responded to treatment, 3 (3.6%) did not maintain their increased T concentration at 4 months. Sex-hormone binding globulin was not observed to change significantly with treatment.

Ejaculated seminal volume and sperm motility were not observed to change with treatment. Sperm concentration significantly increased from 4.7 ± 1.2 × 10^6/mL to 13.1 ± 2.9 × 10^6/mL ($P=.001$), and total motile count increased from 4.6 ± 1.3 × 10^6 to 8.0 ± 3.4 × 10^6 ($P=.008$), as shown in Table 2. In 18 of the 21 patients with oligozoospermia (85.7%), an increase in sperm concentration and total motile count was observed. A commonly used threshold for IUI is 5 × 10^6 total sperm count (16), although other thresholds are also commonly used (17). Nine patients (42.8%) who had a pretreatment total motile count <5 × 10^6 (2.1 ± 0.5 × 10^6) had an increased posttreatment total motile count >5 × 10^6 (8.3 ± 0.7 × 10^6). None of the patients with azoospermia due to spermatogenic dysfunction had return of sperm in the ejaculate, and none of the patients with cryptozoospermia demonstrated significant increase in their sperm count. Eleven patients with azoospermia due to spermatogenic dysfunction underwent microsurgical testicular sperm extraction, and sperm was retrieved in eight (72.7%). The remaining 17 azoospermic patients elected not to undergo surgery or were lost to follow-up.

In a subgroup of 21 oligozoospermic patients with pre- and posttreatment semen analyses, changes in total motile count were not observed to correlate with either pre- or posttreatment T, bioavailable T, or E_2, nor were observed to correlate with the pretreatment T/E_2 ratio. Improvement in total motile count correlated with observation of an increased posttreatment T/E_2 ratio ($P<.0001$), by both absolute and relative increase, even after controlling for age and FSH concentration. Table 3 demonstrates correlations between the percentage of change in total motile count and various hormonal parameters.

Normozoospermic patients did not demonstrate a significant change in their semen parameters ($P=.64$ and $P=.35$ for sperm concentration and total motile count, respectively), nor changes in semen parameters correlated with posttreatment changes in the T/E_2 ratio ($P=.27$, adjusted $R^2 = 0.051$).

Anastrozole treatment was discontinued in 8 patients (9.3%). Two patients reported joint and tendon pain and swelling in their limbs. Other reported side effects in a single patient included decreased libido, irritability and depression, bilateral breast tenderness, ocular pruritus gradually turning into ocular pain, and dry mouth. Another patient demonstrated a paradoxical increase in E_2. One patient reported transient bilateral ankle swelling, which resolved while continuing treatment.

DISCUSSION

Spermatogenesis is a highly regulated process involving complex multifactorial hormonal control. Although the significance of androgens for spermatogenesis is well established, E is increasingly recognized as an important regulator involved in this process (8). Estrogen receptors and the aromatase enzyme are found in both germ cells and somatic cells in the testis, allowing for both production of and modulation by E_2. Estradiol has a significant effect on the growth, development, and function of Leydig cells. It inhibits aromatase activity in Sertoli cells in a paracrine fashion and promotes maturation of spermatogonia (6). Estradiol also affects germ cells in an autocrine fashion, whereby low concentrations of E_2 putatively enhance germ cell production by the inhibition of apoptosis (6). Finally, the lack of functional aromatase results in dysfunctional spermatogenesis (8, 18).

Yet high concentrations of E may be detrimental to spermatogenesis (7). Estrogen exerts negative feedback on the hypothalamus and pituitary, lowering concentrations of FSH and LH and resulting in diminished spermatogenesis (19, 20). Estrogen also plays a major inhibitory local role in the testis. It has been observed to be involved in down-regulation of spermiogenesis-related genes and spermatocyte apoptosis through E receptors 1 and 2 (5). In addition, E displays an inhibitory effect on both Leydig and Sertoli cells (6). Further support of a possible impact of excess aromatase activity on spermatogenesis was demonstrated by Hammond et al. (21), who reported decreased sperm concentration in obese men to be associated with repeat polymorphism rate of TTTA nucleotides in the aromatase gene correlating with aromatase enzyme activity. It seems that a carefully regulated concentration of E_2 is required for normal spermatogenesis.

Numerous studies have demonstrated that aromatase inhibitors increase T, decrease E_2 concentrations, and increase gonadotropin (LH and FSH) concentrations in elderly men,

TABLE 2

Semen parameters in 21 men with hypoandrogenism and oligozoospermia treated with anastrozole.

	Baseline		At 4 mo		
Parameter	**Mean ± SE**	**Median (IQR)**	**Mean ± SE**	**Median (IQR)**	***P* value**
Volume (mL)	2.56 ± 0.22	2.5 (1.7–3.3)	2.32 ± 0.25	2.2 (1.6–3)	NS
Concentration (×10^6/mL)	4.7 ± 1.2	2 (1.5–7.3)	13.1 ± 2.9	7 (4.4–19.5)	.001
Motility (%)	39.9 ± 5	41.5 (24–55)	40.5 ± 4.8	38.5 (29–54)	NS
Total motile count (×10^6)	4.6 ± 1.3	2.8 (0.7–6.6)	8 ± 3.4	8.1 (3.3–12.1)	<.01

Note: Values are mean ± SE or median (interquartile range).

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TABLE 3

Correlation of total motile count percentage change with hormonal parameters in a subgroup of 21 men with hypoandrogenism and oligozoospermia.

	Linear regression			Age- and FSH-adjusted linear regression		
Predictor	Regression coefficient (95% CI)	Adjusted R^2	*P* value	Regression coefficient (95% CI)	Adjusted R^2	*P* value
Pretreatment total T	1.48 (−1.67, 4.63)	−0.002	NS			
Pretreatment BT	2.94 (−3.35, 9.22)	−0.002	NS			
Pretreatment E_2	−1.9 (−19, 15.2)	−0.052	NS			
Posttreatment total T	0.15 (−1.39, 1.69)	−0.053	NS			
Posttreatment BT	0.28 (−3.18, 3.75)	−0.069	NS			
Posttreatment E_2	−4.13 (−14.1, 5.87)	−0.014	NS			
Change in total T	−0.18 (−1.67, 1.31)	−0.052	NS			
Change in total BT	−0.48 (−3.84, 2.89)	−0.064	NS			
Pretreatment T/E_2 ratio	19.8 (−60.5, 100.2)	−0.04	NS			
Posttreatment T/E_2 ratio	14.3 (8.8, 19.8)	0.618	<.0001	14.5 (7.9, 21.1)	0.598	<.0001
Absolute change in T/E_2 ratio	13.8 (8.15, 19.5)	0.586	<.0001	14.2 (7.5, 21)	0.578	<.0001
% Change in T/E_2 ratio	0.8 (0.35, 1.25)	0.419	<.01	0.77 (0.22, 1.31)	0.374	<.01

Note: BT = bioavailable T; CI = confidence interval.

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obese men, and young hypoandrogenic and/or subfertile men (10–14,22,23). Our results are in agreement with these previous findings. Testosterone concentrations doubled as E_2 concentrations nearly halved, and gonadotropin concentrations increased significantly. Less than 5% of patients did not respond to anastrozole with these intended hormonal changes.

Despite the fact that aromatase inhibitors have been prescribed for decades in the treatment of subfertile men, there exists a relative paucity of studies evaluating the effect of aromatase inhibitors in this population, specifically in regard to changes in semen parameters in hypoandrogenic oligozoospermic men. As early as 1981, in an uncontrolled study, Vigersky and Glass investigated the effects of testolactone (1 g/d) in 10 oligospermic men (10). These authors reported an increase in serum T, a decrease in serum E_2, and a significant increase in the androgen-to-E ratio. Eighty percent of men treated demonstrated an increase in sperm count, and there was no change observed in sperm motility. More recently, Pavlovich et al. (11) reported a significantly different T/E_2 ratio of 6.9 in 63 subfertile patients compared with 14.5 in fertile controls. In a subset of 12 patients with oligozoospermia an increase of semen parameters after testolactone therapy was observed. These authors proposed a threshold T/E_2 ratio of 10, representing the lowest 20th percentile of fertile subjects in the control group. An expanded analysis of patients from this study compared 101 subfertile men treated with anastrozole vs. 74 treated with testolactone and demonstrated an improved T/E_2 ratio in both groups (12). A subset of 25 patients with oligozoospermia in the anastrozole group as well as the 12 men in the testolactone group demonstrated increase in their semen parameters. Saylam et al. (13) prospectively investigated the effect of letrozole in 27 infertile men with a decreased T/E_2 ratio and reported increases in semen parameters after 3 months of therapy. Similarly, in a prospective, nonrandomized study, Gregoriou et al. (14) compared anastrozole with testolactone in subfertile men with a T/E_2 ratio <10 (14 vs. 15 patients, respectively), reporting improvement both in hormones and semen parameters, with no significant difference between the two medications. These previous studies indicate that there may be a subgroup of subfertile patients with a treatable endocrinopathy.

Two studies provide alternative results. In a randomized, controlled trial assigning 50 oligozoospemic men to testolactone 2 g daily or placebo for 8 months, followed by crossover for 8 months, Clark and Sherins (24) observed no increase in semen parameters. Helo et al. (25) randomized 24 hypoandrogenic patients to either CC or anastrozole and observed no increase in semen parameters. However, these studies did not evaluate a relevant and similar patient population. The subjects in Clark and Sherins' study (24) had normal T concentrations and likely T/E_2 ratios above the threshold of 10 because the mean T and mean E_2 in the placebo and testolactone initiation arms were 479 ± 50 ng/dL and 27.6 ± 2.3 ng/dL, and 521 ± 6.0 ng/dL and 23.2 ± 1.8 ng/dL, respectively. Furthermore, E_2 and T concentrations did not change with treatment during the trial. In the Helo et al. study (25), the authors aimed to compare the efficacy of CC vs. anastrozole in increasing T concentrations, and the men in their study were not oligozoospermic, with baseline sperm concentration 32.7 ± 12 × 10^6/mL. A reasonable conclusion from these studies is that men who are not hypoandrogenic or who have abundant sperm may not benefit from treatment with aromatase inhibitors.

It is important to note that the definition of "low" T/E_2 ratio as <10:1 is based on the 20th centile of the Cornell cohort. Until further collaborative research is done, we believe the 10:1 ratio should be viewed as a guide, rather than a "hard" cutoff. Although most of our patients had a T/E_2 ratio ≤10, our study population represents a more generalized clinical subset, including patients with previous adverse reaction to CC who may still benefit from oral medication. In the present study, 85% of men with oligozoospermia demonstrated significant increase in sperm concentration and total motile count after treatment with anastrozole. More than one-third of the oligozoospermic patients became eligible for IUI considering a total motile count threshold of 5 × 10^6 (16,

17), and these patients could consequently choose an alternative to IVF. We can expect the pretreatment hormones not to correlate with semen parameters, because this was a selected group of men with low T/E ratio. Importantly, however, increased total motile count significantly correlated with the posttreatment change in the T/E_2 ratio in patients with oligozoospermia. Patients who achieved a higher posttreatment T/E_2 ratio had a greater increase in their total motile count, whereas changes in T or E_2 alone did not correlate with total motile count change. In contrast, normozoospermic patients did not demonstrate a significant change in their semen parameters or changes in semen parameters correlated with changes in the T/E_2 ratio.

Unlike previous studies (13, 14, 26, 27) that reported a return of sperm to the ejaculate in azoospermic patients treated with an aromatase inhibitor, in the present study all 28 azoospermic patients remained without sperm 4 months after anastrozole treatment. Eight cryptozoospermic patients also did not demonstrate a significant increase in semen parameters. The surgical sperm retrieval rate in these azoospermic men with spermatogenic dysfunction was 72.7%. Ramasamy et al. (28), addressing a specific population of azoospermic patients, have shown therapy including anastrozole to result in a 1.4-fold increased sperm retrieval rate in patients with Klinefelter syndrome. However, Schlegel (9) estimated it would require more than 700 patients to evaluate the benefit of aromatase inhibitor in improving sperm retrieval rates in a more general population of men with azoospermia due to spermatogenic dysfunction. The question of whether anastrozole treatment improves the odds of sperm retrieval remains unanswered. We believe that our results support the role of aromatase inhibition therapy in increasing the T/E_2 ratio in the subgroup of oligozoospermic hypoandrogenic subfertile men presenting with a low T/E_2 ratio.

As a rule, in the present study anastrozole treatment was safe and well tolerated. Anastrozole was discontinued in less than 10% of the patients. Side effects usually occurred early into therapy, and all were reversible, subsiding after discontinuing the medication. Notably, two of the eight patients experiencing side effects suffered from arthralgia, and possibly arthritis. A word of caution should be mentioned regarding the possible risk of decreased bone density due to anastrozole treatment (23, 29). This is especially true in a population of men with hypoandrogenism, who are more prone to having decreased bone density to begin with. The significance of E in the male physiology is gradually unfolding. The optimal concentrations of E_2 in men are largely unknown, but low levels of E_2 may be related to increased bone resorption and decrease in bone mass density (29). Notably, 54% of our patients had posttreatment E_2 concentrations ≤ 20 pg/mL. Consequently, long-term skeletal safety remains an issue of concern, and it is important to consider performing bone density scans in patients treated with aromatase inhibitors for longer periods of time.

Our study shares similar limitations with previous studies of aromatase inhibitor therapy in subfertile men. First, T levels were measured once, relying on enzyme immunoassays, which may reduce the reliability of the diagnosis of hypogonadism. Second, we did not have data on patients' body mass index, which may have aided to better define our cohort. Last, this was a retrospective, uncontrolled study, and is consequently subject to selection bias and regression to the mean. We aimed to minimize these biases by selecting the greatest pretreatment total motile count. The observation of a strong correlation between increase in total motile count and increase in the T/E_2 ratio argues against but does not eliminate an effect of regression to the mean.

Despite its limitations, the present study adds to a small but growing body of knowledge on the use of aromatase inhibitors to treat male infertility. We have demonstrated that the magnitude of the increase of the T/E_2 ratio was correlated with increase in bulk seminal parameters in hypoandrogenic oligozoospermic men, supporting a role for aromatase inhibition in this select patient population. Our results also imply that E_2 concentrations should be considered in the evaluation and treatment of subfertile men with oligospermia. Although resource intensive, we hope that future prospective, randomized, controlled studies may study aromatase inhibitor therapy in a selected group of hypoandrogenic men with low T/E_2 ratios and oligozoospermia, because they may prove to share a treatable endocrinopathy.

In conclusion, the majority of men with hypoandrogenism and low T/E_2 ratio responded to anastrozole treatment with improved endocrine parameters. A subset of oligozoospermic men were observed to significantly improve their sperm parameters after 4 months of treatment. In that subset the increase in the total motile count was correlated with the change in the T/E_2 ratio, which argues for a physiologic effect of treatment.

REFERENCES

1. Lipshultz LI, Howards LI, Niederberger CS, editors. Infertility in the male. 4th ed. Cambridge, UK: Cambridge University Press; 2009.
2. Sussman EM, Chudnovsky A, Niederberger CS. Hormonal evaluation of the infertile male: has it evolved? Urol Clin North Am 2008;35:147–55.
3. Jarow JP, Zirkin BR. The androgen microenvironment of the human testis and hormonal control of spermatogenesis. Ann N Y Acad Sci 2005;1061:208–20.
4. Patel DP, Brant WO, Myers JB, Zhang C, Presson AP, Johnstone EB, et al. Sperm concentration is poorly associated with hypoandrogenism in infertile men. Urology 2015;85:1062–7.
5. Dumasia K, Kumar A, Deshpande S, Sonawane S, Balasinor NH. Differential roles of estrogen receptors, ESR1 and ESR2, in adult rat spermatogenesis. Mol Cell Endocrinol 2016;428:89–100.
6. Schulster M, Bernie AM, Ramasamy R. The role of estradiol in male reproductive function. Asian J Androl 2016;18:435–40.
7. Carreau S, Bouraima-Lelong H, Delalande C. Estrogen, a female hormone involved in spermatogenesis. Adv Med Sci 2012;57:31–6.
8. Carreau S, Lambard S, Delalande C, Denis-Galeraud I, Bilinska B, Bourguiba S. Aromatase expression and role of estrogens in male gonad: a review. Reprod Biol Endocrinol 2003;1:35.
9. Schlegel PN. Aromatase inhibitors for male infertility. Fertil Steril 2012;98:1359–62.
10. Vigersky RA, Glass AR. Effects of delta 1-testolactone on the pituitary-testicular axis in oligospermic men. J Clin Endocrinol Metab 1981;52:897–902.
11. Pavlovich CP, King P, Goldstein M, Schlegel PN. Evidence of a treatable endocrinopathy in infertile men. J Urol 2001;165:837–41.

12. Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. J Urol 2002; 167:624–9.
13. Saylam B, Efesoy O, Cayan S. The effect of aromatase inhibitor letrozole on body mass index, serum hormones, and sperm parameters in infertile men. Fertil Steril 2011;95:809–11.
14. Gregoriou O, Bakas P, Grigoriadis C, Creatsa M, Hassiakos D, Creatsas G. Changes in hormonal profile and seminal parameters with use of aromatase inhibitors in management of infertile men with low testosterone to estradiol ratios. Fertil Steril 2012;98:48–51.
15. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72.
16. Merviel P, Heraud MH, Grenier N, Lourdel E, Sanguinet P, Copin H. Predictive factors for pregnancy after intrauterine insemination (IUI): an analysis of 1038 cycles and a review of the literature. Fertil Steril 2010;93:79–88.
17. Group ECW. Intrauterine insemination. Hum Reprod Update 2009;15: 265–77.
18. Robertson KM, O'Donnell L, Jones ME, Meachem SJ, Boon WC, Fisher CR, et al. Impairment of spermatogenesis in mice lacking a functional aromatase (cyp 19) gene. Proc Natl Acad Sci U S A 1999;96:7986–91.
19. Finkelstein JS, O'Dea LS, Whitcomb RW, Crowley WF Jr. Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab 1991;73:621–8.
20. Raven G, de Jong FH, Kaufman JM, de Ronde W. In men, peripheral estradiol levels directly reflect the action of estrogens at the hypothalamo-pituitary level to inhibit gonadotropin secretion. J Clin Endocrinol Metab 2006;91: 3324–8.
21. Hammoud AO, Griffin J, Meikle AW, Gibson M, Peterson CM, Carrell DT. Association of aromatase (TTTAn) repeat polymorphism length and the relationship between obesity and decreased sperm concentration. Hum Reprod 2010;25:3146–51.
22. Loves S, Ruinemans-Koerts J, de Boer H. Letrozole once a week normalizes serum testosterone in obesity-related male hypogonadism. Eur J Endocrinol 2008;158:741–7.
23. Burnett-Bowie SA, Roupenian KC, Dere ME, Lee H, Leder BZ. Effects of aromatase inhibition in hypogonadal older men: a randomized, double-blind, placebo-controlled trial. Clin Endocrinol (Oxf) 2009;70:116–23.
24. Clark RV, Sherins RJ. Treatment of men with idiopathic oligozoospermic infertility using the aromatase inhibitor, testolactone. Results of a double-blinded, randomized, placebo-controlled trial with crossover. J Androl 1989;10:240–7.
25. Helo S, Ellen J, Mechlin C, Feustel P, Grossman M, Ditkoff E, et al. A randomized prospective double-blind comparison trial of clomiphene citrate and anastrozole in raising testosterone in hypogonadal infertile men. J Sex Med 2015;12:1761–9.
26. Cavallini G, Beretta G, Biagiotti G. Preliminary study of letrozole use for improving spermatogenesis in non-obstructive azoospermia patients with normal serum FSH. Asian J Androl 2011;13:895–7.
27. Cavallini G, Biagiotti G, Bolzon E. Multivariate analysis to predict letrozole efficacy in improving sperm count of non-obstructive azoospermic and cryptozoospermic patients: a pilot study. Asian J Androl 2013;15:806–11.
28. Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN. Successful fertility treatment for Klinefelter's syndrome. J Urol 2009;182: 1108–13.
29. de Ronde W, de Jong FH. Aromatase inhibitors in men: effects and therapeutic options. Reprod Biol Endocrinol 2011;9:93.