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Invited Review

Article subject: Male Endocrinology

Alternatives to testosterone replacement: testosterone restoration (TRES)

Andrew McCullough

Department of Urology, Albany Medical College, Albany, New York

Correspondence: Dr A McCullough (amccullough@communitycare.com)

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Abstract

The European Male Aging Study has demonstrated that the hypogonadism of male aging is predominantly secondary. Theoretically with appropriate stimulation from the pituitary, the aging testis should be able to produce eugonadal levels of testosterone. Treatment strategies for the treatment of late onset hypogonadism have focused on replacement with exogenous testosterone versus restoration of endogenous production. The purpose of this article is to review existing peer reviewed literature supporting the concept of restoration of endogenous testosterone in the treatment of late onset hypogonadism.

Keywords: secondary hypogonadism, clomiphene citrate, aromatase inhibitor, enclomiphene

The decrease of testosterone (T) levels with aging has been long since observed [1,2]. Longitudinal studies have clearly documented the decline in T with aging.[3,4] Consequences of low T levels in the human male and the benefit of the normalization of T levels value have been well established. [5] The etiology of late onset hypogonadism (LOH) has been attributed to a decrease in the secretion of hypothalamic and pituitary gonadotropins and a decrease in Leydig cell numbers and responsiveness in the aging male. [6] Yet, the European Male Aging Study (EMAS) has characterized 85 % of hypogonadal men as secondary, i.e. the testes are being insufficiently stimulated by the hypothalamic-pituitary axis.[7]

The risk benefit ratio of testosterone replacement therapies has recently been questioned. Three widely publicized studies (two large retrospective studies and one small prospective) have brought into doubt the cardiovascular safety of testosterone replacement [8-10] despite prior studies suggesting that testosterone replacement might be beneficial to the cardiovascular system.[11,12] In the words of the recently convened FDA advisory panel "these studies do not provide conclusive evidence of increased cardiovascular risk associated with the use of testosterone therapy"[13]. The European Pharmacovigilance Risk Assessment Committee also did not find that current evidence supported the purported association between testosterone replacement and an increased cardiovascular risk. [14]

For decades, the only FDA approved treatment for hypogonadism has been T replacement (TREP). Current guidelines for approvals are all based on T restoration to normalize T levels and not symptomatic improvement. [15] Barring major lifestyle changes, men diagnosed with hypogonadism will require treatment for life, not unlike another highly prevalent condition, type II diabetes. Yet, the treatment of type 2 diabetes is not universally insulin replacement but either oral medication to increase insulin sensitivity or insulin secretion. Fifty-eight percent of diabetics are on oral hypoglycemics with only 12% of diabetics on replacement therapy with insulin. Why is the only recommended therapy for hypogonadism replacement, particularly since 85% of men are secondarily hypogonadal? These are not men with absent

gonadotropins but men with inappropriately low gonadotropins for the low levels of T. Is there another possible therapeutic strategy other than replacement? Can we "restore" T production in the aging male by stimulation of the testes? Current exogenous therapies are fraught with the potential of abuse, possible testosterone transfer to other parties[16,17], erythrocytosis[18,19], induction of infertility by pituitary suppression[20], gynecomastia from hyper-estrogenism[21], the morbidity of a lifetime of intramuscular injections or testosterone pellet insertions and the expense of proprietary applications.[22] The purpose of this article is to explore the current status of testosterone restorative (TRES) therapies. The reader should be warned that none of the discussed therapies are FDA approved.

Human Chorionic Gonadotropins (HCG)

HCG is purified from the urine of pregnant women or through recombinant technology.[23] HCG was discovered 80 years ago and has been commercially available since 1932. It has been used in women to promote the final stages of follicular maturation and progression of the immature oocyte in assisted reproductive strategies. In a retrospective uncontrolled trial of 13 young infertile men with profound hypogonadotropic hypogonadism, HCG was used to induce and maintain spermatogenesis by increasing intra-testicular testosterone production. After induction of spermatogenesis with HCG and human menopausal gonadotropin, in the third phase of the study, men were treated with maintenance HCG instead of T replacement. Though T levels were maintained, prolonged treatment with HCG alone resulted in a decrease in sperm concentrations and testicular volumes over the HCG/HMG phase. Estradiol levels were not reported in the study.[24] Jarow demonstrated the benefit of combined use of low dose HCG in 29 young men (250-500 iU every other day for 3 weeks) with normal reproductive physiology treated simultaneously with exogenous IM T (200mg weekly) and escalating doses of HCG (0,125,250and 500 iU). Whereas intratesticular T was suppressed to 5% of baseline value on T alone, with concomitant administration of 250 iU of HCG intratesticular T levels were maintained at baseline throughout the study. [25] In a retrospective review of 4 years of hypogonadal men presenting to the Baylor andrology clinic, Hsieh reported on 26 men, concerned about their fertility, who were treated simultaneously with HCG treatment and exogenous T (19 IM T 200mg per week and 7 with transdermal gel) for an average of 6.2 months. Despite a mean post-treatment level T level of 1055 ng dl^{-1} seminal parameters (count, motility and morphology) did not significantly change during the periods of observation. Serum gonadotropins were not reported. [26] The Baylor study demonstrated the ability of low dose HCG to maintain spermatogenesis despite the administration of exogenous T. HCG has been used to rescue spermatogenesis in previous anabolic steroid abusers. Lipshultz et al[27] reported the successful treatment of an azoospermic anabolic steroid abuser with HCG

Late onset hypogonadism (LOH) is probably secondary to senescence of central and peripheral endocrine axis. Nonetheless Kaufman demonstrated comparable pituitary LH surges to LHRH stimulation in 10 elderly monks (mean age 72) and 10 young men (mean age 33). He proposed that the aging pituitary is able to respond to gonadotrophs despite a decreased baseline amplitude of the LHRH pulses from the hypothalamus.[28] Handelsman demonstrated that LOH could be treated with HCG. He conducted a 3 month randomized double blind placebo controlled trial of bi-weekly 5000 iU units of HCG in 40 men with a primary endpoint of a 20% increase in muscle strength. The average age was 67 and baseline T was 320 ng dl^{-1}. All men were "healthy, ambulatory, and community-dwelling". The only entry criterion was a T level less than 420ng dl^{-1}. Labs were checked monthly and at one month after termination. Ultrasonographically determined testicular volume was assessed before, at the end of study and one month later. Even though a significant improvement in muscle strength was not demonstrated, a tremendous amount of valuable information was obtained. Lean body mass was improved. T, and E-2 levels increased 145% (778 ng dl^{-1}) and 157% (89 pg ml^{-1}) respectively while gonadotropins plummeted and testicular volumes decreased significantly. Two men had T levels above 1000 ng dl^{-1} and three men developed nipple tenderness. Despite the effectiveness of HCG in the normalization of serum T in older men, the decrease in testicular volume, suppression of gonadotropins and a uniform supraphysiological increase in E-2 levels give pause for concern for long term use as monotherapy in LOH. Larger long term safety and efficacy trials are needed.[29] As most of testicular volume is comprised of seminiferous tubules, the finding of testicular size reduction is in stark contrast to the low dose HCG/IM T Hsieh study which revealed preservation of fertility. Seminal parameters were not reported by Handelsman and testicular volumes were not reported by Hsieh.

Clomiphene Citrate (CC)

Like HCG, CC was originally designed for female infertility. Approved by the FDA in 1967 it has since become an inexpensive generic drug. It is a selective estrogen receptor modulator comprised of a 38%/62% racemic mixture of cis and trans isomers, zuclomiphene and enclomiphene, respectively.[30] It has antagonistic effects on the estrogen receptors in the hypothalamus and the pituitary thereby increasing endogenous gonadotropin releasing hormones, LH and FSH. It ability to increase LH in men was recognized as early at 1968 [31] As with all SERMs organ estrogen agonistic effects are also possible. In a study aimed at using CC challenges to diagnose hypogonadotropic hypogonadism, Paulsen demonstrated significant increases in LH,FSH and T in normal older men taking 50 mgs of CC twice a day.[32] Sherins et al[33] were able show the CC was able to block the LH and FSH suppression that occurs with exogenous T and estrogen administration, thus demonstrating that estrogen was the primary inhibitory hormone on GnRH , LH and FSH. (**Figure 1**) Over the ensuing decades, CC was used to increase male fertility with mixed results. Though an increase in T and estrogen level was consistently demonstrated, no consistent effect of seminal parameters or pregnancy rates was observed. A 6 month multicenter international placebo controlled study cast doubts on the efficacy of CC on idiopathic male infertility. It is important to realize that in the international study, the infertile population was eugondal with the mean baseline T levels of 481ng dl^{-1}. Well controlled studies in the hypogonadal infertile male are lacking, despite the high prevalence of secondary hypogonadism in this group of men.

Tenover et al[34] looked at an 8 week trial of CC (50mg BID) in 5 healthy older and 5 young eugonadal men (mean age 73 *vs* 29; mean baseline T 518 *vs* 498) and demonstrated that older men both increased LH and FSH and T and E-2. Though levels of T were significantly lower in the older group, the levels achieved in both groups were well above levels that are achieved with many current day exogenous treatments. (**Figure 2a** and **2b**) Lim observed normalization of testosterone levels in 5 hypogonadal uremic men with uniform increase in libido, sexual potency, and a general sense of well-being using 100 mg of CC daily for as long as 12 months. The normalization of T continued for 4-5 months after discontinuation of therapy. Plasma estradiol levels were elevated at baseline and did not change significantly from baseline.[35]

Guay el al[36] challenged 21 older men with erectile dysfunction and secondary hypogonadism with 50 mg CC bid for 7 days and normalized their T, demonstrating that at that at least in the short term, the concept of testosterone restoration was possible in older men. He then expanded the concept with an eight week double blind placebo controlled crossover study in older men (mean age 62) with secondary hypogonadism and erectile dysfunction (documented with nocturnal penile tumescence scan (NPT). No improvement was seen in NPT or sexual function questionnaires in the group as a whole. When the study population was split between younger and older groups (mean age 53 and 66respectively) in a post hoc analysis, not surprisingly, the differences between the treatment groups with the sexual function questionnaires and NPT testing achieved statistical significance. The older men were more likely to have “end organ” disease” refractory to hormonal manipulation. This was the first demonstration that CC could not only normalize T levels in SHGD but result in symptomatic improvement. [37] Guay then began treating men in his practice with SHGD with CC (50 mg) three times a week. He reported an observational series of 173 men with ED and SHGD treated for 4 months. The diagnosis of ED was based on self-report and not a validated questionnaire and a placebo arm was lacking. The outcome was measured as “responder” to treatment (successful intercourse >75% of the time), partial responder (successful intercourse 50%-75% of the time) and non-responder. As in his previous studies, LH, FSH and free testosterone levels increased. Sexual function improved in 75% and did not change in 25%. Age and vascular co-morbidities negatively affected the response rates. [38]

Taylor et al in an observational study compared the biochemical efficacy of CC to exogenous gel treatment (TRT) in 104 men (65 CC *vs* 39 on TRT). The groups were not strictly identical but demonstrated comparable increases in testosterone with a 182 $ monthly savings in the CC group. PSA levels and HCT did not significantly change in follow up (23 months)[39] Moskovic demonstrated an excellent chemical response in a younger cohort of 29 men (mean age 44) followed for three years on CC 25 mgs every other day. In addition, despite an unusually high percentage of men with altered bone mineral density at baseline (75%) BMD normalized at one year in 25%. No improvement in BMD was observed after the first year. Though estradiol increased significantly no gynecomastia or breast tenderness occurred. No side effects were reported.[40]

The efficacy of CC in relieving the symptoms of hypogonadism is often anecdotally reported as being inferior to exogenous therapy without the support of randomized double blind studies. Katz et al retrospectively looked at symptom relief with CC (25mg every other day) in 86 young (mean age 29) hypogonadal men, most of whom were

presenting for infertility (57 %) over a 4 year period at a Sloan Kettering andrology practice. The men were followed for a mean of 19 months. Surprisingly the median number of positive baseline responses on the androgen deficiency in aging males (ADAM) questionnaire was 5 that dropped to 2. These "generally very healthy" young men started at a mean T level of 192 ng dl^{-1} and increased their T to 485 (despite a target treatment level of 550 ng dl^{-1}). The symptoms that showed significant increases included "decreased libido, lack of energy, decreased life enjoyment, sad/grumpy, decreased sports performance".[41] The lack of a placebo arm weakens the strength of the study. Further support of the efficacy of CC in relieving hypogonadal symptoms comes from a retrospectively gathered observational comparative study from Baylor by Ramasamy. In examining the effect of CC vs replacement therapy on hypogonadal symptoms, no significant differences were seen in between T injections, T gels or CC. T levels were highest with injections (1104 ng dl^{-1}) *vs* CC (504 ng dl^{-1}) or the gels (412 ng dl^{-1}).[42] The lack of a difference in symptom relief supports the concept that symptom relief may be tied to a threshold level that is achieved with TRES and TREP. Unfortunately pre-treatment quantitative ADAM scores (QADAM) were not reported and the QADAM has not been fully psychometrically validated.

Recently there has been interest in the trans isomer of CC (EC). Distinct differential pharmacokinetics of the two isomers have been demonstrated.[43] Though the Cmax, and Tmax were comparable, the AUC for the isomers was dramatically different after a single dose administration of 50 mgs of CC in women with polycystic ovaries. At 456 hours, ZC was detected in 9/9 patients vs 1/9 for EC.[44] The half-life of EC is 7-8 hrs. [45] EC was evaluated in an early proof of concept randomized, open-label, fixed dose, active-control (7EC and 5 exogeous gel), two-center phase IIB study in 12 men with secondary hypogonadism treated previously with topical testosterone. After T discontinuation of exogenous T, T levels in both groups average 165ng dl^{-1}. After treatment T levels increased in both groups to over 540 ng dl^{-1} but decreased to baseline after cessation of treatment suggesting that the hypothalamic testicular axis reverts to its pretreatment state and continued therapy is necessitated. Whereas sperm counts were increased in all men on EC at 6 months only 2 of 5 of gel patients increased their sperm concentrations to over 20 million per ml. GTP increased only in the EC arm.[46] In follow up clinical trials, safety and clinical efficacy were comparable to a gel preparation while preserving sperm counts. Sperm counts were decreased in the men treated with gels. (**Figure 4**) Side effects were comparable to CC. The most significant adverse events were hot flushes (10%), visual disturbances headaches, nausea and vomiting. Aside from the hot flushes, all events occurred in less than 5% of the study population. [47] The ease of use, low side effect profile, therapeutic efficacy and preservation of fertility, make EC if approved an attractive therapeutic alternative to standard TREP.

Aromatase Inhibitors

Aromatase is a cytochrome P450 enzyme responsible for the biosynthesis of estrogen from testosterone. Its evolutionary importance in bone metabolism is underscored by its ubiquitous presence in the vertebrate phyla and absence in the non vertebrate phyla. [48] The importance of estrogen in men is demonstrated in men with congenital aromatase deficiency. Male aromatase deficiency syndrome caused by a mutation in the CYP19, is characterized by elevated testosterone, LH, FSH, absent estradiol levels, osteopenia, failure of epiphyseal plate closure and tall stature. [49] Aromatase has been found in brain, testes, adipose tissue, muscle, hair, bone and vascular tissue.[50]

Aromatase inhibitors are classifies as steroidal or non steroidal. First generation aromatase inhibitors such as aminogluthethamide are non specific and caused suppression of the production of adrenal steroids, necessitating adrenal steroid replacement. The third generation non steroidal aromatase inhibitors such as anastrozole and letrozole are highly specific and potent for aromatase and are well tolerated. Adrenal steroid replacement is not necessary. Non-steroidal third generation aromatase inhibitors have been used to lower estrogens in women with metastatic breast cancer since the early 2000's. Much is known about the pharmacokinetics and safety of the AI's in women. The recommended dose is 1 mg a day. Estradiol levels are suppressed to 0.8 pg ml^{-1}, with 70% of suppression achieved in 24 hours and 80% by 14 days. Estrogen synthesis suppression is maintained for up to 6 days after discontinuation of therapy. The drug is rapidly absorbed with a bioavailability of 85% and a peak serum level achieved at 2 hours from ingestion in the fasting state. The pharmacokinetics are linear over a dose of 1 mg to 20mg. Steady state levels are 3-4 times that of a single dose and are reached at 7 days. Hepatic metabolism and renal excretion is 85% and 10% respectively. No age related effects were seen, no dose adjustment was needed in renal impairment or mild hepatic cirrhosis.[51] A pharmacokinetics study of a I mg of anastrozole in 20 healthy male volunteers revealed a 100%

bioavailability, T_{max} at 1.2 hrs, a $T_{1./2}$ of 42 hours and a C_{max} of 1000 ng ml^{-1}.[52] In women the most common side effects in these women with metastatic breast cancer (>10%) were hot flashes, nausea ,asthenia, pain, headache, back pain , bon pain, increased cough, dyspnea, pharangitis and peripheral edema. [51]

Though safe and effective in this very ill female population it has been associated with 11% incidence of osteoporosis also seen in congenital male aromatase deficiency syndrome and estrogen receptor deficiency.[49,53] Fears about bone demineralization with AI's have limited their long term use in men. Are these fears justified? In a one year randomized placebo controlled trial of anastrozole in 69 hypogonadal men there were no statistically significant changes in bone mineral densities from baseline in the treatment group. The posterior-anterior spine BMD decreases seen (1.7%), only achieved statistically significance because the placebo group increased (0.7%). The standard deviation on baseline determinations of posterior-anterior spine BMD was 17% making the solitary observation of a 1.7% decrease in BMD of questionable clinical significance. (**Figure 5-6**) Trabecular BMD assessed by quantitative computerized tomography and multiple measured highly sensitive bone turnover markers (BTM) demonstrated no significant differences from the placebo group.[54] In another placebo controlled study of 42 obese hypogonadal men followed for 6 months using another aromatase inhibitor, letrozole, no significant differences in BMD or BTM were seen. Finally, in the 5 year comparator trial of anastrozole and tamoxiphene in 189 women with breast cancer, most of the significant bone loss occurred within the first two years. The authors state *"no woman with a normal BMD at baseline became osteoporotic at 5 years and only those women with a T-score of less than -1.5 are at risk of developing osteoporosis".*[55] In fact, there have never been any studies published in men that demonstrate a loss of bone mineral density with aromatase inhibitor use in men.

How is that possible? Unlike the male deficiency syndrome, estradiol is not eliminated with AI's but is reduced. Published male trials reveal a 50% to 65 % reduction in serum estradiol levels in men on AI's to a level of 17-34 p ml^{-1}. [54,56,57] Testosterone levels in hypogonadal men are increased by as much as 144%. [54,57] . Approximately 20 % daily estradiol is produced in the testes, accounting for 60% the circulating estradiol.[58,59] At steady state serum levels, the serum molar concentration of aromatase is 4-10 times that of serum testosterone levels in a hypogonadal man effectively blocking aromatase conversion peripherally. Nonetheless in the testes where the testosterone concentrations reach levels up to 200 times the serum,[60] the AIs will stochiometrically never successfully compete with endogenous testosterone and estrogen will always be produced. With significantly increased testosterone levels and modest decreases in circulating estrogen levels, it is not surprising that the markers of bone turnover markers have not shown any changes in placebo controlled trials.

In an early placebo controlled study of the steroidal aromatase inhibitor, testolactone, in eugonadal men with idiopathic infertility, testosterone was found to increase modestly with a no change in seminal parameters or estradiol levels.[61] Pavlovich looking at testolactone in hypogonadal infertile men demonstrated a 30 % increase in testosterone, a decrease in estradiol levels and an improvement in seminal parameters. As with the SERM's the data on the use of aromatase inhibitors to improve male fertility is equivocal. What is not arguable is the effect of the non steroidal aromatase inhibitors on the pituitary secretion of gonadotropins, testosterone increase and estrogen decrease. Veldhius in a placebo controlled study administered anastrozole to 20 older men (60-76) and measured LH pulsativity, testosterone increase and estrogen decrease. He demonstrated that a 5 day course of anastrozole significantly increased LH, testosterone, decreased estradiol but demonstrated age associated regulatory changes in the pituitary-gonadal axis secondary to estrogen-dependent defects in feedback control.[62] These findings are consistent with the EMAS study demonstrating that most of the LOH is secondary and should respond to strategies to increase pituitary gonadotropins.

There are several placebo controlled studies on aromatase inhibitors to increase testosterone. All have shown significant improvements in testosterone levels comparable or better than those found in comparable topical testosterone studies. [54,57,63] (**Figure 7-9**) Adverse effects in the longest study (one year) have been minimal. Hematocrits, liver functions and urinary symptoms were not changed whereas PSA increased significantly but the changes were not clinically significant (PSA< 3). Changes in bone mineral density were discussed above.

Summary

In view of the EMAS studies secondary hypogonadism accounts for over 85% of late onset hypogonadism. Ample evidence exists for a deficiency in GTP stimulation with the older men and the ability of the testes to respond to

increased GTP production. We currently have several generic medications that accomplish an increase in GTP and normalization of serum testosterone with a favorable side effect profile. Though shown to be efficacious and well tolerated in a number of trials, none of the restorative strategies are FDA approved and caution must be advised in their off label use. Hopefully future trials will be undertaken to establish the long term efficacy and safety these restorative therapies. Early clinical trials of the compound enclomiphene are encouraging and hopefully will lead to a change in paradigm from TREP to TRES.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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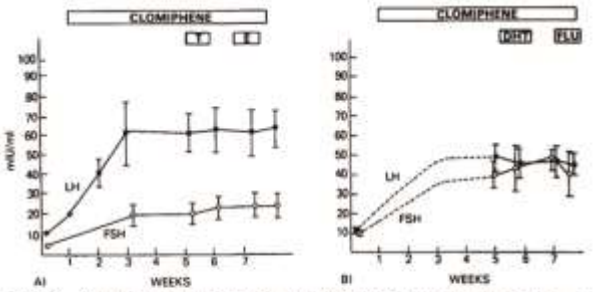

FIG. 3. Serum levels of LH (●) and FSH (○) in two groups of normal men receiving clomiphene citrate (100 mg orally twice a day for 8 weeks). A, During week 5, T (15 mg/day) and, in week 7, E (90 μg/day) were infused for 4 days into group I. B, Group II received DHT (7.0 mg/day) in week 5 and fluoxymesterone (FLU; 10 mg orally every 6 h) during week 7, each for 4 days.

Figure 1 Inhibitory effect of CC on Sex Steroid Inhibition of GTP[33]

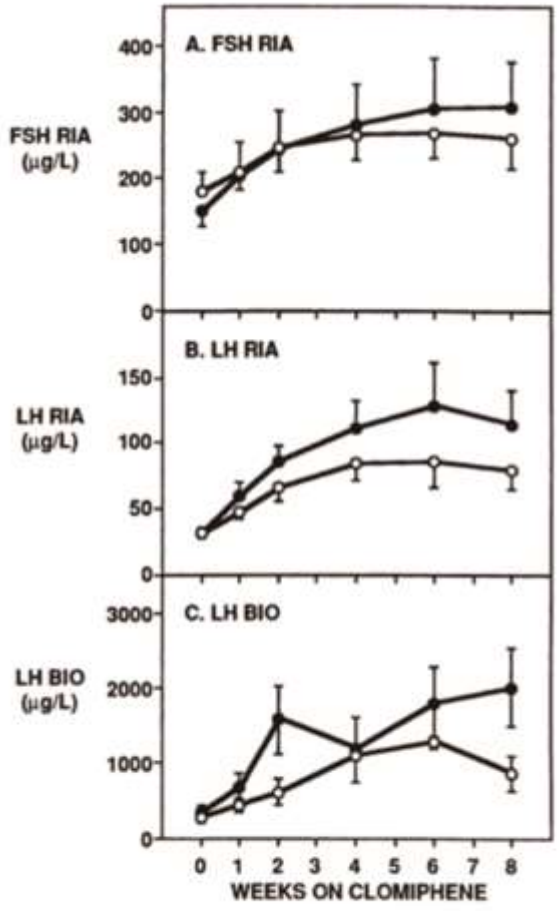

FIG. 1. Effect of clomiphene citrate administration on serum levels (mean ± SE) of FSH RIA (A), LH RIA (B), and LH BIO (C) in five young adult (filled circles) and five elderly (open circles) healthy men.

Figure 2a Effect of CC on GTP in older and younger men [34]

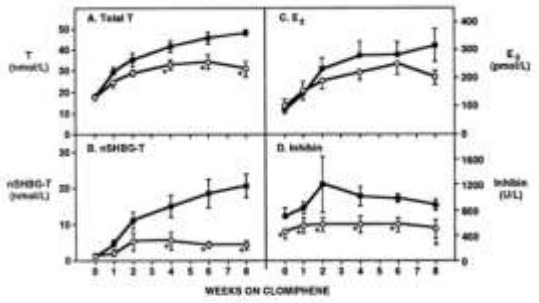

Figure 2b Effect of CC on sex hormones in older and younger men [34]

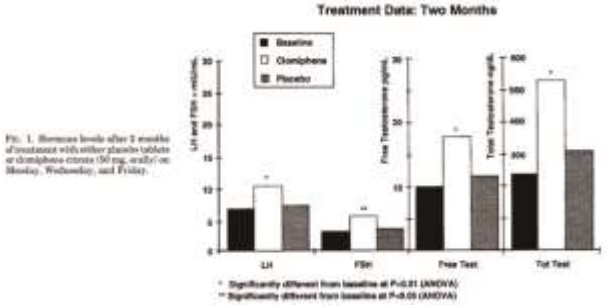

Figure 3 Placebo contolled study of clomiphene in older men [37]

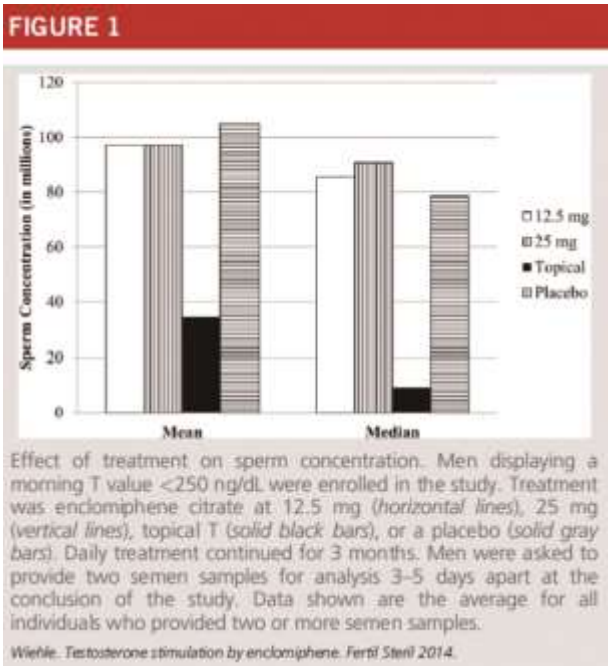

Figure 4 Comparison of the effecty of enclomiphene and gels on sperm counts [64]

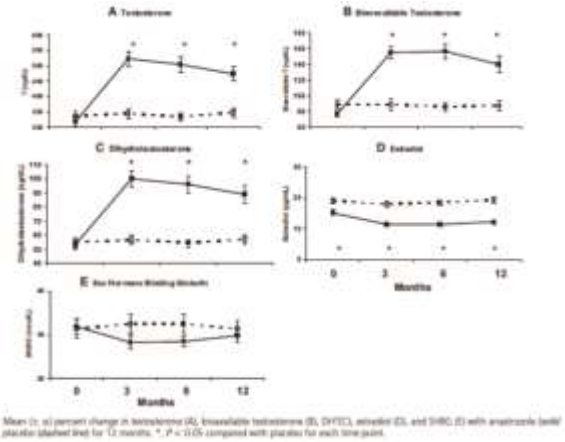

Figure 5-Placebo controlled study of the effect of aromatase inhibitor on sex hormones and bone mineral density[54]

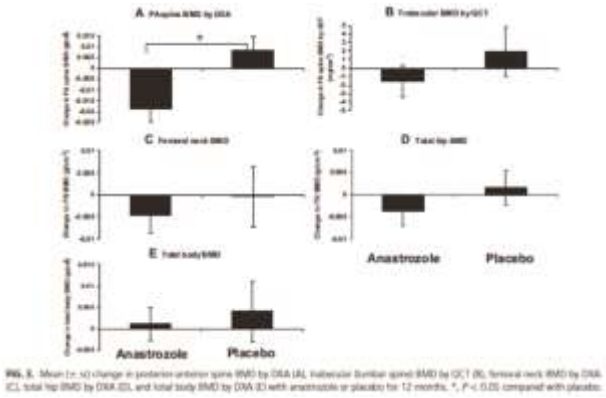

Figure 6 Placebo controlled study of the effect of AZ on sex hormones and bone mineral density[54]

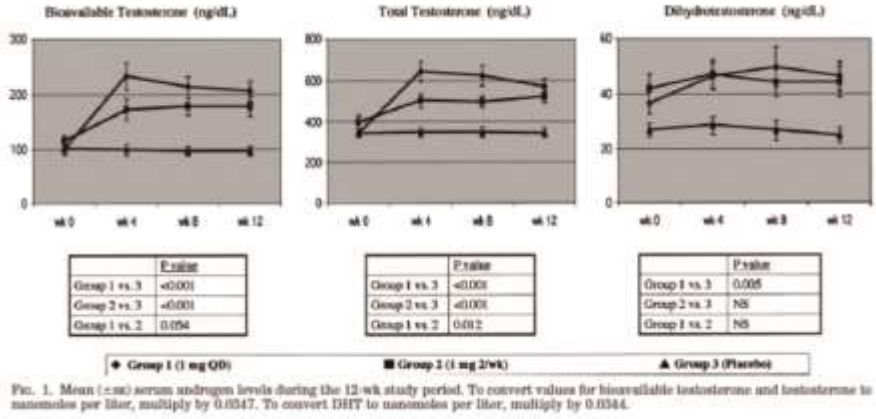

Figure 7 Placebo controlled study of AZ :effect on androgens[63]

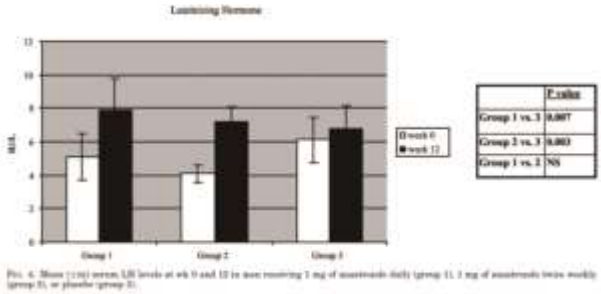

Figure 8- Placebo controlled study of AZ: Effect on LH [63]

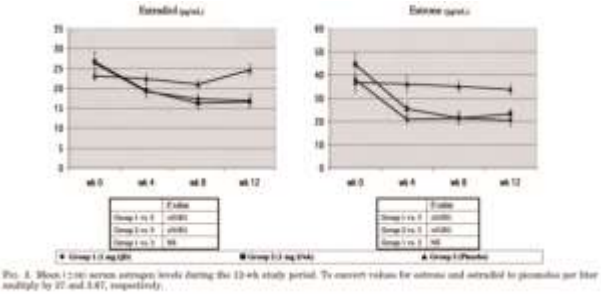

Figure 9-Placebo controlled study on AZ: Effect on estrogenic hormones [63]