9

The use of testosterone as a male contraceptive

JOHN K. AMORY MD
Acting Instructor in Medicine

WILLIAM J. BREMNER MD, PhD
Professor and Vice-Chairman in Medicine
Chief, Medical Service

Department of Veterans Affairs-Puget Sound Health Care System, Population Center for Research in Reproduction and Department of Medicine, University of Washington, Seattle, WA, USA

Testosterone functions as a contraceptive by suppressing secretion of the pituitary gonadotropins luteinizing hormone and follicle stimulating hormone. Low levels of these hormones decrease endogenous testosterone secretion from the testis and deprive developing sperm of the signals required for normal maturation. Interference with sperm maturation causes a decline in sperm production and can lead to reversible infertility in men, raising the possibility that testosterone could be utilized in a commercially available contraceptive. To this end, testosterone has been studied alone and in combination with either gonadotropin releasing hormone analogues or progestins in efforts to improve its contraceptive efficacy. In this chapter, we will review efforts to use testosterone to create a safe, convenient, efficacious contraceptive method for men.

Key words: contraceptive agents, male, pharmacology; contraceptive agents, male, administration and dosage; testosterone, analogues and derivatives; testosterone, pharmacology; oligospermia, chemically induced; sperm count.

Since the introduction of the progestin–oestrogen oral contraceptive for women in the early 1960s, there has been interest in developing a similarly safe and effective means of contraception for men. Given the success of the endocrine approach in the creation of the women's pill, researchers have attempted to use hormones to provide contraception for men. Testosterone and its derivatives are the most widely tested hormones. Testosterone can be used alone or in combination with a second agent to impair spermatogenesis and thereby function as a contraceptive.

In women, the progestin–oestrogen oral contraceptive prevents ovulation by limiting the production of the pituitary gonadotropins follicle stimulating

Correspondence should be addressed to Dr Bremner.

Vol. 12, No. 3, October 1998
ISBN 0–7020–2463–5
0950–351X/98/030471 + 14 $12.00/00

hormone (FSH) and luteinizing hormone (LH). Similarly, in men, exogenous testosterone impairs spermatogenesis by an inhibition of LH and FSH production by negative feedback at the level of the pituitary. Normally, LH stimulates the production of testosterone by Leydig cells in the testis, while FSH acts on Sertoli cells in the seminiferous tubules of the testis to facilitate sperm maturation. In men, a blockade of LH production shuts down the testicular production of testosterone. A complete absence of testosterone, however, is impractical in the long term as testosterone is necessary to prevent the signs and symptoms of hypogonadism and maintain the normal male phenotype. In addition to its importance in sexual behaviour and libido, testosterone plays a role in the maintenance of muscle mass, mineral and bone metabolism, kidney function and haemopoiesis. The absence of FSH, or a blockade of the FSH receptor, has a deleterious effect on sperm counts as well, but mature sperm capable of fertilization persist (Matsumoto and Bremner, 1989).

Many substances, including oestrogen, progestins, gonadotropin analogues and, of course, testosterone can provide the negative feedback necessary to depress pituitary gonadotropin production, but only testosterone can maintain the male phenotype. Testosterone, however, possesses some limited ability to maintain spermatogenesis, even in the absence of FSH and LH. This fact has bedeviled researchers in this area for decades; testosterone, necessary to prevent hypogonadism, stimulates spermatogenesis.

Attempts at a solution to this dilemma are the subject of this review. In the broadest terms, researchers have approached this problem by attempting to either:

1. provide just enough testosterone to decrease LH and FSH production and prevent hypogonadism, but not so much as to stimulate spermatogenesis;
2. use a second agent such as a progestin or gonadotropin releasing hormone (GnRH) analogue intended to decrease the production of LH and FSH while also administering sufficient testosterone to prevent hypogonadism.

In this chapter, we will first discuss the nomenclature and preparations of testosterone commonly used, as well as the side-effects and drawbacks of such preparations when used as potential male contraceptives. Then, in the following three sections, we will follow this research as it progressed through three phases: the first in which testosterone was used alone; a second phase in which (GnRH) agonists and antagonists were given in combination with testosterone to further decrease the production of LH and FSH; and the third and current phase of research, which involves the combination of testosterone with a progestin.

TESTOSTERONE: NOMENCLATURE AND PREPARATIONS

Testosterone is the principal androgen in men and the pro-hormone for the production of two classes of steroids: dihydrotestosterone (DHT) and

oestrogens. DHT is 5α-reduced from testosterone and acts as the intracellular mediator of many androgen actions. Oestrogens, mostly in the form of oestradiol, serve to enhance some androgenic effects and block others (see below).

The administration of testosterone itself is impractical because, when given orally or by injection, it is promptly degraded by the liver. Therefore, the testosterone available for human use has been modified chemically to prolong its action. The most common modification is esterification at the 17-hydoxyl group with any of several carboxylic acids. This decreases the polarity of the molecule, making it more soluble in the lipid vehicles used for injection, and hence slows the release of the injected steroid into the circulation (Wilson, 1990). These esters, such as testosterone enanthate (TE), a long-acting ester that can be dosed weekly by intramuscular injection, are hydrolysed to testosterone before the hormone acts, and drug activity can therefore be measured in an assay for testosterone. The side-effects of this regimen include minor injection site irritation, oily skin and acne, and increases in lean body mass. No problematic changes in libido or aggressiveness have been found.

Orally active preparations are currently available; most of these hormones are alkylated at the 17-carbon position. 17-Alkylation slows catabolism by the liver, allowing oral dosing; however, these compounds cause liver damage and are not considered safe for long-term use in oral contraceptives (Bagatell and Bremner, 1996). Research into sustained delivery systems for testosterone has been ongoing. Testosterone buciclate, a synthetic ester given by depot injection, maintains physiological androgen levels for up to 3 months in hypogonadal men (Behre and Nieschlag, 1992). Testosterone undecanoate, an ester that is absorbed via lymphatics and therefore escapes first-pass hepatic metabolism (Coert et al, 1975), can be given orally twice a day; it can also be used by injection, from which it maintains serum testosterone level for 4–6 weeks in hypogonadal men (Zhang et al, in press). In addition, research into injectable steroid polymer microparticles or fused crystalline testosterone implants reveals a similar ability to maintain testosterone levels in hypogonadal men (Bhasin et al, 1992; Handelsman et al, 1992). In addition, newer androgens are currently being evaluated (see below).

TESTOSTERONE CONTRACEPTIVE TRIALS: GENERAL CONSIDERATIONS

In assessing the efficacy of a potential male contraceptive, it is important to determine what level of sperm inhibition is necessary to achieve infertility; however, this is not precisely known. The range of normal sperm counts can vary widely, from 20 to 200 million sperm per ml of ejaculate. The absence of sperm in the ejaculate, a condition termed azoospermia, renders fertilization impossible and is therefore the ultimate goal of male contraception. Unfortunately, azoospermia has not been achieved reliably in all normal men using existing hormonal techniques. To date, all

studies report some subjects who sustain a severe but incomplete reduction of their sperm counts, a condition called oligospermia. There is good evidence that sperm counts below 3 million sperm per ml ejaculate are associated with decreased rates of pregnancy (World Health Organisation, 1996). This 'severe oligospermia' considerably decreases the chances of conception and is considered to be a intermediate goal for male contraceptive research.

Additional factors important in the design of a contraceptive include time until onset of action and method of administration. Hormonal contraceptives do not incapacitate existing sperm: they block sperm production. Since sperm take an average of 72 days to reach maturity, it is probable that any contraceptive based on manipulation of the hormonal axis will be associated with some delay in the onset of full contraceptive potency. The lack of a safe, orally active testosterone means that existing regimens rely on frequent injections, but injections are problematic for many men. The need for injections is a significant drawback to current efforts to find a widely appealing method, and the need for sterile needles and syringes may limit its applicability in less developed countries, where contraception is most needed. Last, it is important to consider ethnic differences in interpreting the results of contraceptive trials. For unknown reasons, volunteers in Asia were more susceptible to the steroid suppression of spermatogenesis than were men studied in Europe, North America and Australia (Handelsman et al, 1995). This difference is not solely a function of body mass and may point to either increased sensitivity to lowered FSH and LH levels, or to a decreased conversion of testosterone to DHT in the testis by differing isoforms of 5α-reductase (Anderson et al, 1996). While the mechanism remains to be elucidated, these differences are important in interpreting the results of various clinical trials and make extrapolation of the data to different populations difficult.

CONTRACEPTIVE TRIALS

Testosterone alone

Testosterone given at slightly supraphysiological levels can suppress both FSH and LH production and simultaneously replace the androgen deficit caused by decreased LH levels. Low levels of LH also lead to decreases in *intratesticular* testosterone as the Leydig cell production of testosterone falls. This decrease in intratesticular testosterone is of crucial importance as normal local concentrations of testosterone and DHT are necessary for spermatogenesis (Morse et al, 1973).

As early as the 1930s, the administration of testosterone was shown to suppress sperm counts (Heckel, 1939); however, the first systematic studies of testosterone as a contraceptive date from the 1970s. Several small studies were reported in 1977 (Steinberger and Smith, 1977a,b; Swerdloff et al, 1979) using TE alone given by intramuscular injection. In these trials

of Caucasian men, more than half of the subjects were rendered azoospermic, and most of the others became severely oligospermic. As expected, the onset of azoospermia was at around 72 days, and the recovery of normal sperm counts occurred 3–4 months after testosterone was discontinued.

Based on these initial encouraging results, two large, multicentre trials of TE were conducted by the World Health Organization (WHO) (1990, 1996). The first study enrolled 271 subjects who were given weekly doses of 200 mg TE intramuscularly for a 6-month induction phase. Sixty-five per cent of these men achieved azoospermia, and an additional 30% were rendered severely oligospermic. The fertility of the azoospermic men was then tested in a 12-month efficacy phase. Of the 119 couples who became azoospermic, continued the injections and used no other form of birth control, only one pregnancy occurred. This pregnancy rate of 0.8 pregnancies per 100 person-years demonstrates that, in men rendered azoospermic, TE is an effective contraceptive. Patients discontinued involvement with the study mainly because of regimen failure and dislike of the injection schedule.

The second WHO study examined the fertility of both the men who became azoospermic and the men who achieved severe oligospermia on the TE regimen (World Health Organization, 1996). A total of 399 men were enrolled in this study. Of these, all but eight (2%) became severely oligospermic or azoospermic. In terms of fertility, there were no pregnancies fathered by the men who became azoospermic; in men whose sperm counts suppressed to below 3 million per ml, fertility was reduced to 8.1 pregnancies per 100-person years. The combined fertility rate for oligospermic and azoospermic men was 1.4 per 100-person years. Therefore, the overall failure rate (including the men who failed to suppress to oligospermia) was 3.4%, for an overall contraceptive efficacy of 96.6%.

This research demonstrated that testosterone is safe, fully reversible and effective as a contraceptive in the majority of men. Drawbacks to testosterone-alone methods are, however, apparent. While effective in those who achieve azoospermia, some men fall to suppress below 3 million sperm per ml and therefore presumably remain fertile. In addition, the necessity of weekly intramuscular injections is a deterrent. Twenty-five per cent of patients in the second WHO study discontinued involvement for personal or medical reasons, or because of a dislike of the injection schedule. Last, high-dose testosterone has been shown to decrease serum high-density lipoprotein (HDL) cholesterol, which could contribute to accelerating atherosclerosis (Bagatell et al, 1994; Meriggiola et al, 1995). These failings have led to two additional avenues of research: (a) the addition of a second agent, either a GnRH analogue or a progestin; and (b) attempts to improve the characteristics of testosterone administration.

Testosterone and GnRH analogues

The decapeptide structure of GnRH was first discovered in 1971 (Matsuo et al, 1971). Since then, many analogues with both agonist and antagonist

properties have been synthesized. Compounds with agonist properties cause an initial stimulation of gonadotropin secretion. After 2–3 weeks of use, the pituitary loses its ability to respond to GnRH, causing a marked fall in gonadotropin levels. This finding led to a flurry of research into using GnRH agonists in male contraception. At least 12 trials using three different GnRH agonists with testosterone have been reported (reviewed in Cummings and Bremner, 1994). The results of these studies were, however, disappointing, with roughly 25% of men achieving azoospermia, a third achieving severe oligospermia, and the remainder suppressing only partially to levels under 30 million sperm per ml, a degree of suppression that is inadequate for use as a reliable contraceptive. These results have led to the almost complete abandonment of trials using of GnRH agonists as male contraceptives.

Some synthetic GnRH analogues have been shown to possess potent antagonist properties and suppress the production of FSH and LH. These GnRH antagonists can suppress FSH and LH production within hours of administration, and their inhibition of the gonadotropins is more complete than can be produced by agonists. The side-effects include erythema and occasional burning sensations at the site of injection, and local pruritus. Some subjects develop non-tender, subcutaneous nodules at the injection site; these resolve in a few weeks.

At least three human trials have been conducted using the GnRH antagonist Nal-Glu with testosterone. The first two trials showed promise, seven out of eight subjects in one study achieving azoospermia by 6–10 weeks of treatment (Pavlou et al, 1991; Tom et al, 1992). A third trial, however, demonstrated no difference in azoospermia when compared with TE alone (Bagatell et al, 1993). The time required to reach azoospermia was roughly 7–10 weeks. Normal sperm counts returned in roughly the same amount of time, demonstrating the reversibility of this approach.

GnRH antagonists have significant drawbacks. Since they are peptides, they are expensive to make and must be injected subcutaneously to avoid degradation in the intestine; also, most have very short half-lives. Furthermore, it seems possible that testosterone, when given in conjunction with GnRH antagonists, blunts the antagonist's ability to impair spermatogenesis. Since more testosterone is required to initiate spermatogenesis than to maintain the process when already active (Harris et al, 1977), it has been suggested that the administration of an antagonist alone for 70–100 days could be used to induce azoospermia before testosterone was provided to prevent hypogonadism. To date, this approach has been tried mainly in primates and seems more successful than combined initial administration.

While these trials showed promise, it is clear that the use of currently existing GnRH antagonists in the long term is impractical. Current research is focusing on either the synthesis of an orally active, non-peptide antagonist, or longer-acting antagonists that could be given in a few injections to initiate spermatogenic arrest. Such compounds could then be tested in combination with sufficient long-term testosterone administration to prevent hypogonadism.

Testosterone and progestins

The idea of using a progestin with testosterone to synergistically block the production of pituitary gonadotropins was first tested in the 1960s, mostly using depot medroxyprogesterone acetate (DMPA). Over 35 studies were carried out and showed that progestins possessed the ability to inhibit LH and FSH secretion in men (reviewed in Schearer et al, 1978). Furthermore, it has been suggested that progestins may also have a direct suppressive effect on spermatogenesis (Fotherby et al, 1972; Meriggiola and Bremner, 1997).

In five studies conducted by the Population Council, 100 volunteers were given monthly injections of DMPA in combination with 100–250 mg TE intramuscularly for 4–16 months (Alvarez-Sanchez et al, 1977; Brenner et al, 1977; Frick et al, 1977a,b; Melo and Coutinho, 1977). These combinations were able to induce azoospermia in half of the subjects, and some degree of oligospermia in most others, but the contraceptive efficacy of these combinations was poor. Nine couples conceived while on therapy despite the simultaneous use of other contraceptives (Barfield et al, 1977). Doubling the amount of TE in the regimen did not improve its efficacy (Faundes et al, 1981). However, in one study of 20 Indonesian men, azoospermia was induced in all the subjects (Pangkahila, 1991). Recently, DMPA has been shown to increase the contraceptive efficacy of implanted T pellets (Handelsman et al, 1996). Drawbacks to this class of 17-hydoxyl progestational agent were significant; patients experienced weight gain, transient decreases in HDL cholesterol and gynaecomastia.

Following these first studies, compounds with fewer side-effects, such as the potent oral progestin levonorgestrol (LNG), were tested as an adjuvant agent for male contraception. Two early studies of LNG used 250–500 μg per day LNG given orally combined with low doses of TE (200 mg intramuscularly per month). This combination was chosen to minimize testosterone-driven spermatogenesis and maximize the LNG-mediated inhibition of FSH and LH. In the first study, 13 men were enrolled, but seven withdraw for various reasons. Sperm counts were depressed below 5 million per ml in half of the subjects, but none reached azoospermia (Foegh et al, 1980). The second study showed similar results, with three out of five subjects in the 500 μg LNG group achieving counts of fewer than 6 million sperm per ml (Fogh et al, 1980). No toxicological side-effects or changes in libido, potency or blood coagulation parameters were detected in either study. While the results were disappointing, these studies did demonstrate that LNG could serve as a useful and well-tolerated adjunct to testosterone. It is also clear, however, that for a combined testosterone–LNG method to succeed, more frequent dosing of testosterone would be required.

Recently, a randomized, controlled trial of LNG (500 μg orally daily) with frequent dosing of TE (100 mg intramuscularly per week) showed that the LNG–TE combination was superior to TE alone in terms of azoospermia (67% versus 33%) by 6 months (Bebb et al, 1996). In addition, the total achieving severe oligospermia or azoospermia was 94% in the LNG–TE group compared with 61% of the TE-alone group. The time to onset of azoospermia was more rapid in the LNG–TE group than among those

receiving TE alone (a mean of 9 versus 15 weeks). Drawbacks to the LNG–TE regimen included greater weight gain and decreases in HDL cholesterol when compared with the TE-alone group. Encouraged by this result, the same research group is evaluating lower doses of LNG, in the hope that side-effects can be minimized while contraceptive efficacy is maintained.

Anti-androgens with progestational effects such as cyproterone acetate (CPA) have also been tested as potential male contraceptives. CPA is a synthetic steroid with progestational and anti-androgenic properties (Neumann and Topert, 1986). CPA functions as an anti-androgen by blocking the binding of testosterone and DHT to androgen receptors. CPA thus interferes with androgen-dependent spermatogenesis in the testis and suppresses gonadotropins at the pituitary by means of its progestational activity (Meriggiola and Bremner, 1997).

The combination of T and CPA was first tried in the early 1980s (Roy, 1985). Then, following the successful use of TE and CPA in monkeys (Lohiya et al, 1987), this combination was re-tried in humans. In a small trial, subjects were divided into three groups of five patients each. The first two groups received CPA at either 50 or 100 mg orally daily, as well as weekly doses of 100 mg TE intramuscularly. The third group received just TE weekly. All men receiving CPA became azoospermic, while only three out of five in the TE-alone group attained azoospermia (Figure 1) (Meriggiola et al, 1996). In addition, the time required to achieve azoospermia in the CPA groups was half that needed in the testosterone-alone group (49 versus 98 days). This result is both surprising and encouraging given the aforementioned 72-day maturation time of sperm, implying that this regimen may suppress partially mature sperm as well as decreasing the generation of new spermatids. Happily, no major adverse side-effects, such as changes in HDL cholesterol, liver function, libido or sexual potency, were noted in this small sample of men. The sole drawbacks noted were slight decreases in body weight and haemoglobin level that were dependent on the dose of CPA.

The same group (Meriggiola et al, 1997) has recently reported on the first oral combination of CPA with testosterone undecanoate. In this study, a lower dose of CPA was used (12.5 mg twice a day) combined with 80 mg testosterone undecanoate twice a day. Of eight subjects, one became azoospermic, five were suppressed below 3 million sperm per ml, and the two remaining subjects were suppressed to 4 and 6 million sperm per ml respectively. It is hoped that alterations in the regimen will lead to more complete and reliable spermatogenic suppression.

Given the positive results from combinations of testosterone and progestins, many researchers now feel that these combinations are the most likely to result in a viable contraceptive method. Current research is focused on finding dose combinations that optimize sperm count suppression in all populations while minimizing side-effects.

NEWER ANDROGENS

Many of the trials discussed have used TE; however, the requirement for

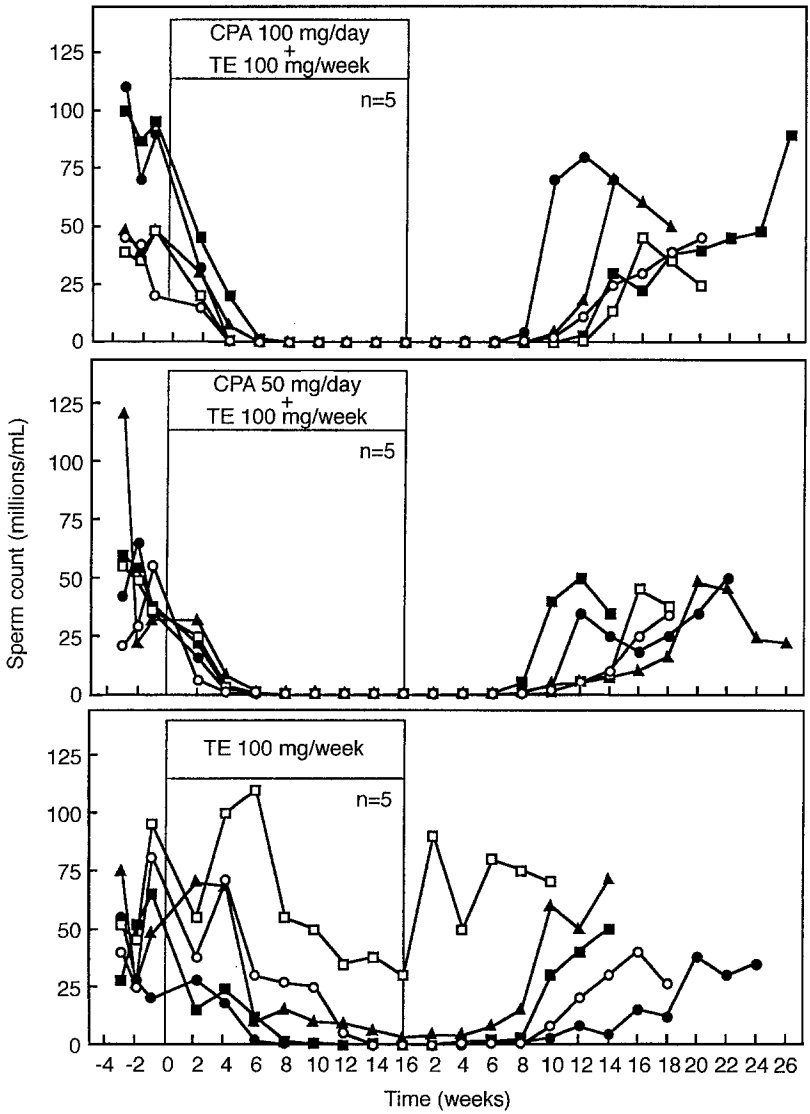

Figure 1. Sperm concentrations in individual subjects during the control period, throughout 16 weeks of hormone administration and during 26 weeks of the recovery phase. Reproduced from Meriggiola et al (1996, *Journal of Clinical Metabolism* **81:** 3018–3023) with permission of the Endocrine Society.

weekly injections is a significant drawback. Longer-acting esters such as 19-nortestosterone (19-NT) have been evaluated as potential substitutes for TE. In addition to its potent androgen effects, 19-NT has 10 times the progestational activity of testosterone and therefore inhibits FSH and LH production to a greater degree than does TE. In early small-scale studies, rates of azoospermia were in the 80–90% range when combined with the

progestin DMPA (Knuth et al, 1989). In addition, the motility and morphology of the sperm in those rendered oligospermic was found to be impaired. A large multicentre trial was conducted by the WHO in Indonesia using weekly injections of either TE or 19-NT in combination with the progestin DMPA. Both groups had greater than 95% azoospermia, showing 19-NT to be at least as effective as TE, with no untoward side-effects (World Health Organization, 1993). It remains to be seen whether this early success can by improved upon in Caucasian populations, perhaps in combination with newer progestins such as LNG or CPA.

A derivative of 19-NT, 7α-methyl-19-nortestosterone (5-MENT), is also of interest. The 7-methylation of this compound prevents 5α-reduction, thus preventing its DHT-like effects (Sundaram et al, 1994). This is of particular importance in spermatogenesis, as it has been recently shown that men who remained oligospermic following TE administration had higher DHT levels (Anderson et al, 1996). In addition, lower levels of DHT may prevent stimulation of the prostate and decrease the theoretical risk of prostatic enlargement after the long-term administration of testosterone. To date, 5-MENT has not been used in contraceptive trials, but it seems a likely candidate for future studies.

RECENT ADVANCES IN MOLECULAR ENDOCRINOLOGY

Recently, several advances have been made in our understanding of oestrogen activity at the molecular level. A second oestrogen receptor has been discovered (Kuiper et al, 1996), and the two oestrogen receptors, ERα and β, can initiate or inhibit transcription depending on the particular gene, promoter and agonist (Paech et al, 1997). These findings could explain the observations that 'anti-oestrogens' such as tamoxifen can have agonist activity in one tissue (endometrium) and antagonist activity in another (breast). Such compounds, more appropriately termed 'selective oestrogen receptor modulators' or SERMs, are coming into clinical use both for the treatment of breast cancer in women and for use as oestrogen replacement therapy in post-menopausal women (Delmas et al, 1997).

Exciting by themselves, these observations also raise the possibility of greater complexity at the level of testosterone hormone binding and transcriptional activation. Is there more than one androgen receptor? Certainly, receptors of non-steroidal hormones, such as thyroid hormone and retinoic acid, are known to have multiple subtypes. To date, however, there is little evidence of such complexity at the level of the testosterone receptor. If found, such a discovery would suggest the creation of a testosterone analogue that could selectively activate certain testosterone-responsive genes while inhibiting others. Such a compound, possibly termed a 'selective androgen receptor modulator' or 'SARM' has the potential to block spermatogenesis and prostatic enlargement while providing for the beneficial effects of androgens.

In addition to a greater understanding of oestrogen's function and gene regulation in women, investigators are now finding that oestrogen has

essential functions in spermatogenesis. An early clue to the importance of oestrogen was the infertility of the oestrogen receptor knockout mouse (Lubahn et al, 1993; Korach et al, 1996). These mice lack functional oestrogen receptors, ERα, but have fully functioning androgen receptors. Their seminiferous tubules are dysfunctional and appear to be unable to maintain a healthy environment for sperm maturation in the epididymis (Hess et al, 1997). *De novo* mutations in oestrogen receptor function and aromatase activity have also been reported in humans (Smith et al, 1994; Morishima et al, 1995). The fertility of these men is undocumented, but if they are infertile, it would imply that oestrogen blockade could be a potential target for the creation of a male contraceptive. Such agents, however, would have to overcome the adverse side-effects of oestrogen blockade, such as osteoporosis, the non-fusion of epiphyseal growth plates and hyperlipidaemia, as in subjects lacking oestrogen function.

CONCLUSION

Testosterone is useful as a contraceptive in human males; however, a regimen with 100% effectiveness has remained elusive. Combinations with GnRH antagonists improve the efficacy of testosterone but are presently impractical for widespread use. Testosterone combinations with progestins appear promising. Ongoing trials with testosterone plus LNG and CPA may offer a usable option for men, but difficulties in testosterone delivery may hinder their use. Recent insights into the molecular regulation of transcription by oestrogen receptors may point to the existence of similar complexity in the androgen receptor and may provide new avenues for the generation of a male hormonally derived contraceptive. A better understanding of the molecular regulation of spermatogenesis will clearly increase the chances of successfully manipulating these systems to create an easily usable, long-term contraceptive for men, and something, finally, to complement the success of the oestrogen–progestin pill for women.

REFERENCES

Alvarez-Sanchez F, Faundes A, Brache V et al (1977) Attainment and maintenance of azoospermia with combined monthly injections of depot medroxyprogesterone acetate and testosterone enanthate. *Contraception* **15:** 635–648.

Anderson RA, Wallace AM & Wu FC (1996) Comparison between testosterone entanthate-induced azoospermia and oligospermia in a male contraceptive study. III. Higher 5α-reductase activity in oligospermic men administered supraphysiological doses of testosterone. *Journal of Clinical Endocrinology and Metabolism* **81:** 902–908.

Bagatell CJ & Bremner WJ (1996) Androgens in men-uses and abuses. *New England Journal of Medicine* **334:** 707–714.

Bagatell CJ, Matsumoto AM, Christensen RB et al (1993) Comparison of a gonadotropin releasing-hormone antagonist plus testosterone versus testosterone alone as potential male contraceptive regimens. *Journal of Clinical Endocrinology and Metabolism* **77:** 427–432.

Bagatell CJ, Heiman JR, Matsumoto AM et al (1994) Metabolic and behavioral effects of high-dose, exogenous testosterone in healthy men. *Journal of Clinical Endocrinology and Metabolism* **79:** 561–567.

Barfield A, Melo J, Coutinho E et al (1977) Pregnancies associated with sperm concentrations below 10 million/ml in clinical studies of a potential male contraceptive method, monthly depot medroxyprogesterone acetate and testosterone esters. *Contraception* **20:** 121–127.

*Bebb RA, Anawalt BD, Christensen RB et al (1996) Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. *Journal of Clinical Endocrinology and Metabolism* **81:** 757–762.

Behre HM & Nieschlag E (1992) Testosterone buciclate (20 Aet-1) in hypogonadal men: pharmacokinetics and pharmacodynamics of the new long-acting androgen ester. *Journal of Clinical Endocrinology and Metabolism* **75:** 1204–1210.

Bhasin S, Swerdloff RS, Steiner B et al (1992) A biodegradable testosterone microcapsule formulation provides uniform eugonadal levels of testosterone for 10–11 weeks in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism* **74:** 75–83.

Brenner PF, Mishell DR, Bernstein GS et al (1977) Study of medroxyprogesterone acetate and testosterone enanthate as a male contraceptive. *Contraception* **15:** 679–691.

Coert A, Geelen J, de Visser J et al (1975) The pharmacology and metabolism of testosterone undecanoate (TU), a new orally active androgen. *Acta Endocrinologica* **79:** 789–800.

*Cummings DE & Bremner WJ (1994) Prospects for new hormonal male contraceptives. *Endocrinology and Metabolism Clinics of North America* **23:** 893–922.

Delmas PE, Bjarnason NH, Mitlak BH et al (1997) Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. *New England Journal of Medicine* **337:** 1641–1647.

Faundes A, Brache V, Leon P et al (1981) Sperm suppression with monthly injections of medroxyprogesterone acetate combined with testosterone enanthate at a high dose (500 mg). *International Journal of Andrology* **43:** 235–245.

Foegh M, Nicol K, Peterson IB & Schou B (1980) Clinical evaluation of long-term treatment with levo-norgestrel and testosterone enanthate in normal men. *Contraception* **21:** 631–640.

Fogh M, Corker CS, McLean H et al (1980) Clinical trial with levo-norgestrel and testosterone oenanthate for male fertility control. *Acta Endocrinologica* **95:** 251–257.

Fotherby K, Davies JE, Richards DJ & Bodin M (1972) Effect of low doses of synthetic progestagens on testicular function. *International Journal of Fertility* **17:** 113–119.

Frick J, Bartsch G & Weiske WH (1977a) The effects of monthly depot medroxyprogesterone acetate and testosterone on human spermatogenesis. I. Uniform dosage levels. *Contraception* **15:** 649–668.

Frick J, Bartsch G & Weiske WH (1977b) The effects of monthly depot medroxyprogesterone acetate and testosterone on human spermatogenesis. II. High initial dose. *Contraception* **15:** 669–677.

Handelsman DJ, Conway AJ & Boylan LM (1992) Suppression of human spermatogenesis by testosterone implants. *Journal of Clinical Endocrinology and Metabolism* **75:** 1326–1332.

*Handelsman DJ, Farley TM, Peregoudov A et al (1995) Factors in nonuniform induction of azoospermia by testosterone enanthate in normal men. World Health Organization Task Force on Methods for the Regulation of Male Fertility. *Fertility and Sterility* **63:** 125–133.

Handelsman DJ, Conway AJ, Howe CJ et al (1996) Establishing the minimum effective dose and additive effects of depot progestin in suppression of human spermatogenesis by a testosterone depot. *Journal of Clinical Endocrinology and Metabolism* **81:** 4113–4121.

Harris ME, Bartke A, Weisz J et al (1977) Effects of testosterone and dihydrotestosterone on spermatogenesis, rete testis fluid, and peripheral androgen levels in hypophysectomized rats. *Fertility and Sterility* **28:** 1113–1117.

Heckel NJ (1939) Production of oligospermia in a man by the use of testosterone propionate. *Proceedings of the Society for Experimental Biology and Medicine* **40:** 658–659.

Hess RA, Bunick D, Lee K et al (1997) A role for estrogens in the male reproductive system. *Nature* **390:** 509–512.

Knuth UA, Yeung C & Nieschlag E (1989) Combination of 19-nortestosterone-hexoxyphenylpropionate (Anadur) and depot medroxyprogesterone-acetate (Clinovir) for male contraception. *Fertility and Sterility* **51:** 1011–1018.

Korach KS, Couse JF, Curtis SW et al (1996) Estrogen receptor gene disruption: molecular characterization and experimental and clinical phenotypes. *Recent Progress in Hormone Research* **51:** 159–186.

Kuiper GG, Enmark E, Pelto-Huikko M et al (1996) Cloning of a novel receptor expressed in rat prostate and ovary. *Proceedings of the National Academy of Sciences of the USA* **93:** 5925–5930.

Lohiya NK, Sharma OP, Sharma RC & Sharma RS (1987) Reversible sterility by cyproterone acetate

plus testosterone enanthate in monkey with maintainence of libido. *Biomedical and Biochemical Acta* **46:** 259–266.

Lubahn DB, Moyer JS, Golding TS et al (1993) Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proceedings of the National Academy of Sciences of the USA* **90:** 11162–11166.

*Matsumoto AM & Bremner WJ (1989) Endocrine control of human spermatogenesis. *Journal of Steroid Biochemistry* **33:** 789–790.

Matsuo H, Baba Y, Nair RM et al (1971) Structure of the porcine LH and FSH releasing hormone. *Biochemistry and Biophysics Research Communications* **43:** 1334–1339.

Melo JF & Coutinho EM (1977) Inhibition of spermatogenesis in men with monthly injections of medroxyprogesterone acetate and testosterone enanthate. *Contraception* **15:** 627–634.

*Meriggiola MC & Bremner WJ (1997) Progestin–androgen combination regimens for male contraception. *Journal of Andrology* **18:** 240–244.

Meriggiola MC, Marcovina S, Paulsen CA & Bremner WJ (1995) Testosterone enanthate at the dose 200 mg/week decreases HDL-cholesterol levels in healthy men. *International Journal of Andrology* **18:** 237–242.

*Meriggiola MC, Bremner WJ, Paulsen CA et al (1996) A combined regimen of cyproterone acetate and testosterone enanthate as a potentially highly effective male contraceptive. *Journal of Clinical Endocrinology and Metabolism* **81:** 3018–3023.

Meriggiola MC, Bremner WJ, Constantino A et al (1997) An oral regimen of cyproterone acetate and testosterone for spermatogenic suppression in men. *Fertility and Sterility* **68:** 844–850.

Morishima A, Grumbach MM, Simpson ER et al (1995) Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *Journal of Clinical Endocrinology and Metabolism* **80:** 3689–3698.

Morse HC, Horike N, Rowley MJ et al (1973) Testosterone concentrations in testes of normal men: effects of testosterone propionate administration. *Journal of Clinical Endocrinology and Metabolism* **37:** 882–886.

Neumann F & Topert M (1986) Pharmacology of antiandrogens. *Journal of Steroid Biochemistry* **25:** 885–895.

*Paech K, Webb P, Kuiper GG et al (1997) Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. *Science* **277:** 1508–1510.

Pangkahila W (1991) Reversible azoospermia induced by an androgen–progestin combination reigmen in Indonesian men. *International Journal of Andrology* **14:** 248–256.

Pavlou SN, Brewer K, Farely MG et al (1991) Combined administration of a gonadotropin-releasing hormone antagonist and testosterone in men induces reversible azoospermia without loss of libido. *Journal of Clinical Endocrinology and Metabolism* **73:** 1360–1369.

Roy S (1985) Experience in the development of hormonal contraceptive for the male. In Asch RH (ed.) *Recent Advances in Human Reproduction*. Rome: Fondazione per gli Studi sulla Reproduzione Umana, pp 95–104.

Schearer SB, Alvarez-Sanchez F, Anselmo J et al (1978) Hormonal contraception for men. *International Journal of Andrology* **2 (supplement):** 680–695.

Smith EP, Boyd J, Frank GR et al (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *New England Journal of Medicine* **331:** 1056–1061.

Steinberger E & Smith KD (1977a) Effect of chronic administration of testosterone enanthate on sperm production and plasma testosterone, FSH and LH levels: a preliminary evaluation of a possible male contraceptive. *Fertility and Sterility* **28:** 1320–1328.

Steinberger E, Smith KD (1977b) Testosterone enanthate: a possible reversible male contraceptive. *Contraception* **16:** 261–268.

Sundaram K, Kumar N & Bardin CW (1994) 7-alpha-methyl-19-nortestosterone: an ideal androgen for replacement therapy. *Recent Progress in Hormone Research* **49:** 373–376.

Swerdloff RS, Campfield LA, Palacios A et al (1979) Suppression of human spermatogenesis by depot androgen: potential for male contraception. *Journal of Steroid Biochemistry* **11:** 663–670.

Tom L, Bhasin S, Salameh W et al (1992) Induction of azoospermia in normal men with combined Nal-Glu gonadotropin-releasing hormone and testosterone enanthate. *Journal of Clinical Endocrinology and Metabolism* **75:** 476–483.

Wilson JD (1990) Androgens. In Gilman AG, Rall TW, Nies AS & Taylor P (eds) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edn, pp 1413–1430. New York: Pergamon Press.

*World Health Organization (1990) Contraceptive efficacy of testosterone-induced azoospermia in normal men. *Lancet* **336:** 995–999.

World Health Organization (1993) Comparison of two androgens plus depot-medroxyprogesterone acetate for suppression to azoospermia in Indonesian men. *Fertility and Sterility* **60:** 1062–1068.

*World Health Organization. Task Force on Methods for the Regulation of Male Fertility (1996) Contraceptive efficacy of testosterone-induced azoospermia and oligospermia in normal men. *Fertility and Sterility* **65:** 821–829.

Zhang G, Gu Y, Wang X et al (1998) A pharmacokinetic study of injectable testosterone undeconoate in hypogonadal men. *Journal of Andrology*.