Oestradiol enhances testosterone-induced suppression of human spermatogenesis

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Introduction

A hormonal male contraceptive must suppress reversibly sperm output to amounts that reliably prevent conception with minimal side-effects (Handelsman, 1999). The feasibility of an effective, safe and practical male contraceptive was established by two landmark World Health Organization (WHO) studies using a prototype androgen-alone regimen involving 670 couples in 10 countries (WHO Task Force on Methods for the Regulation of Male Fertility, 1990, 1996). These studies reinforced the working goal for any hormonal male contraceptive method was to achieve uniform azoospermia to ensure contraception that was reliable enough by modern standards (Trussell and Kost, 1987; Trussell et al., 1990). These studies prompted the development of improved, second-generation male hormonal regimens aiming for better suppression (i.e. universal azoospermia, elimination of semen monitoring) and enhanced delivery to optimize compliance through improved depot hormonal formulations which allow longer and more convenient freedom between dosages.

Various hormonal regimens have shown promise for such development of practical hormonal male contraceptive approaches (Handelsman, 1999). The simplest and most widely used approach has been to use androgen-alone regimens. In addition to the WHO studies using weekly testosterone and oestradiol (WHO Task Force on Methods for the Regulation of Male Fertility, 1990, 1996), depot forms of testosterone such as subdermal testosterone implants (Handelsman et al., 1992) or injectable testosterone buciclate (Behre et al., 1995) or undecanoate (G.Y. Zhang, personal communication) have shown various degrees of spermatogenic suppression, although none attains the goal of producing uniform azoospermia. The genetic and/or environmental reasons for this non-uniformity of spermatogenic suppression remain to be elucidated (Handelsman et al., 1995). In order to enhance the spermatogenic suppression, combination regimens have been developed. These use a second, gonadotrophin-suppressing agent coupled with androgen replacement to replace endogenous androgen secretion which is inevitably abolished. Despite sacrificing simplicity, combination regimens might enhance spermatogenic suppression while reducing mutually the minimally effective doses of both active agents. Effective regimens featuring second agents so far include gonadotrophin-releasing hormone (GnRH) antagonists (Cummings and Bremner, 1994) or synthetic progestins (Patanelli, 1977; Scheer et al., 1978; Handelsman, 1999). The GnRH antagonists are, however, expensive, difficult to formulate, may cause irritation due to histamine release and require large daily doses. Synthetic progestins are available mostly in oral formulations, which entails route-dependent, first-pass hepatic overdosage, and regimens involving concurrent oral and injectable dosing are inherently impractical. Another category of sex steroid not evaluated as a second agent for hormonal male contraception is that of...
oestrogens. Pioneering studies by Ewing and associates in the 1970s demonstrated that the addition of a low dose of oestradiol to testosterone greatly enhanced suppression of spermatogenesis in a wide variety of animals including non-human primates but this concept was never tested in man. The present study was designed to test Ewing’s conjecture, that the addition of a small dose of oestradiol to a depot testosterone formulation is required for maximal suppression of mammalian spermatogenesis in humans.

Materials and methods

Participants
Volunteers were recruited from the general population by advertising in print and broadcast media. Recruitment was aimed at men aged 18–45 years, in good general health with normal clinical and biochemical baseline evaluation (including routine tests of liver, kidney and haematological function), normal reproductive function (including two semen analyses and blood hormone levels). They were required to provide written informed consent agreeing to comply with protocol and were cautioned about competing in elite sports requiring urinary drug screening. Men were excluded if they had known cardiovascular, prostate, reproductive, major psychiatric or other serious medical disorders, a history of drug abuse, were taking any regular medication or had contraindications to minor local surgery or administration of testosterone or oestradiol. In addition, men entering the study after the interim review which allowed a higher oestradiol dose were screened for the factor V Leiden mutation, a naturally occurring mutation causing high risk of venous thromboembolism in young women taking oral oestrogens (Vandenbroucke et al., 1994). The study was approved by the Central Sydney Area Health Service Ethics Review Committee (RPAH zone) and was conducted within National Health and Medical Research Council (NHMRC) guidelines for human experimentation.

Design
The study had a randomized, open-label, parallel group design conducted in a single centre. The primary efficacy end-point was the degree of suppression of sperm output as indicated by the proportion achieving azoospermia and the time-course of sperm concentrations. The secondary efficacy end-points were the time course of plasma reproductive hormones (total and free testosterone, oestradiol, LH, FSH). The safety end-points were spontaneously reported adverse effects, standard physical examination, time course of metabolic indicators of androgen effects [sex hormone binding globulin (SHBG), lipids], prostate-specific antigen (PSA), body composition (weight, bioimpedance analysis), clinical chemistry (renal, hepatic and haematological function) and acceptability (psychometric measures of mood, behaviour and acceptability). Gynaecomastia was defined as glandular enlargement of breast tissue reported spontaneously by participants and/or noted at routine physical examination by study personnel.

Eligible volunteers were randomized into groups receiving either testosterone alone or testosterone plus oestradiol implants as a single dose at the start of the study. The testosterone dose was 600 mg (three 200 mg implants) for all groups. Initially, the oestradiol dosage was 10 mg, which was achieved by bisecting a 20 mg oestradiol implant under sterile conditions immediately prior to implantation. Due to the uncertainty in estimating an appropriate oestradiol dosage for healthy young men for whom no published data were available, an interim summary of efficacy blinded to group assignment was undertaken when half the participants were recruited. As this indicated negligible difference between groups in spermatogenic suppression, the 10 mg oestradiol arm was closed and a third arm using a higher oestradiol dose (20 mg) was opened. In order to retain balance between remaining open arms of the study, further randomization to the testosterone alone and testosterone plus 20 mg oestradiol arms was undertaken using urn randomization (Wei and Lachin, 1988). In this procedure, known as unbalanced randomization, the probability of assignment to either group was variable, according to the current state of imbalance in randomization, rather than fixed. At the time of randomization of each subject, \( P \) (the probability of assignment to the smaller group) was recalculated from its definition as \( 2^{d/(2^d + 1)} \) where \( d \) was the difference in numbers in each groups which it was intended to balance. The subject was then randomized according to the drawing of a random \( P \) value from a uniform \([0,1]\) distribution.

Procedures
All participants underwent a single implantation procedure under local anaesthesia at entry to the study. At that implantation procedure all volunteers received according to their randomization, testosterone 600 mg (three 200 mg implants) alone or together with an oestradiol implant [10 mg or 20 mg implant; supplied by Organon (Australia) Pty Ltd, Sydney, Australia]. Testosterone and oestradiol were administered as implants of fused cylindrical rods (testosterone 12 mm long, 4.5 mm diameter; oestradiol 2.5 mm long, 2.25 mm diameter) of pure crystalline steroid without excipients. Subdermal implantation was performed by skilled operators on the lateral anterior abdominal wall under local anaesthetic. No sutures or antibiotics were required and the biodegradable implants did not require removal.

During baseline evaluation, participants provided two sets of blood and semen samples and during the 12 months of the study the participants provided monthly blood and semen samples for 12 months and underwent body composition (bioelectrical impedance) measurement at months 1–3, 6 and 12.

Assays
Semen samples collected by masturbation after at least 2 days without ejaculation were analysed by standard WHO methods (WHO, 1992).

Plasma samples were stored for measurement in commercial immunoassays for LH (Abbot Axysm, Abbott Laboratories, Abbott Park, IL, USA), FSH (Abbot Axysm), total testosterone (Biomedic Immulite, Diagnostic Products Corp., Los Angeles, CA, USA), oestradiol (Delfia, Wallac Oy, Turku, Finland), SHBG (Biomedic Immulite) and PSA (Delfia). Between-assay coefficients of variation were <10% for all assays. Free testosterone was measured by an in-house centrifugal ultrafiltration method (Handelsman et al., 1990, 1996) with a between-assay coefficient of variation of 12%. Clinical chemistry assays (liver function, renal function and lipids) were undertaken by routine autoanalyser methods (Handelsman et al., 1990, 1996).

Changes in body composition were determined after an overnight fast by measurement of body weight to the nearest 0.1 kg and the percentage fat was determined by bioelectrical impedance analysis using the SEAC Model BIM 3.0 bioimpedence meter implementing the Luksaszi algorithm (Luksaszi, 1987). Using body weight and percentage fat, fat mass and lean body mass were calculated. The reproducibility of the bioimpedence measurements was 5.6% for lean mass and 7.7% for fat mass (pooled within – subject coefficient of variability for 9 people measured on 3 occasions at 3 month intervals).

Data analysis
Continuous variables were analysed by analysis of variance for factorial or repeated measures designs with post-hoc testing by Duncan’s test or by Student’s t-test as appropriate using SAS (SAS...
The overall proportion of men achieving azoospermia was 6/26 (23%) and severe oligozoospermia (≤1 or <3×10⁶ spermatozoa per ml) was 7/26 (27%) but did not differ significantly between the three groups. Oestradiol (pooling both dose groups) appeared to enhance the likelihood of achieving azoospermia [33 versus 9%, odds ratio (OR) 5.0, one-sided 95% confidence interval (CI) 0.5–128] or severe oligozoospermia (40 versus 9%, OR 6.7 95%, CI 0.8–166), although neither confidence interval excluded 1.0 with their width indicating the relatively low power of the study to exclude a difference in proportions (for one-sided α = 0.05, azoospermia 29%, oligozoospermia 45%).

Results

Participants

Thirty-five volunteers were screened to find 26 eligible men who completed screening and were randomized to enter the study. One man was excluded on the basis of factor V Leiden mutation. Ultimately, 11 men entered the testosterone alone group (T), seven the testosterone plus oestradiol 10 mg (TE10) and eight the testosterone plus oestradiol 20 mg (TE20) arms. The participants were well matched in age, height, body weight, lean body weight, fat mass, testis and body size and baseline hormones (Table I). The reliability of follow-up was high with only nine isolated missed visits (2.5% of scheduled visits) and the five men (three for personal reasons, two due to job transfers away from Sydney) who discontinued prematurely all completed at least 6 months in the study.

Sperm output

By chance, baseline sperm concentration was higher in the TE20 group (Table I). The nadir sperm concentration differed significantly between groups but these could not be solely due to difference in baseline sperm concentrations as the decrement in sperm concentrations (from baseline to nadir) was significantly greatest in the TE20 group. Oestradiol treatment significantly enhanced the suppression of sperm output (treatment P = 0.004) in a dose-dependent manner for the first 6 months as illustrated by the different time course (treatment×time interaction, P < 0.0001) of sperm concentrations (Figure 1).

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Table 1. Physical characteristics, hormone concentrations and sperm concentrations of the 26 participants in this study

<table>
<thead>
<tr>
<th>Group</th>
<th>Testosterone (nmol/l)</th>
<th>Oestradiol (nmol/l)</th>
<th>SHBG (nmol/l)</th>
<th>Sperm concentrations (×10⁶/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Nadir</td>
<td>Mean</td>
<td>Semen</td>
</tr>
<tr>
<td>T</td>
<td>45 ± 15</td>
<td>14 ± 9</td>
<td>23 ± 1</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>TE10</td>
<td>46 ± 15</td>
<td>10 ± 9</td>
<td>20 ± 1</td>
<td>0.92</td>
</tr>
<tr>
<td>TE20</td>
<td>47 ± 15</td>
<td>14 ± 9</td>
<td>18 ± 1</td>
<td>0.93</td>
</tr>
</tbody>
</table>

P = 0.003.
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Figure 1. Plot of sperm concentration ($\times 10^6$ spermatozoa/ml) against time in months for men treated with 600 mg testosterone alone (T group), 10 mg oestradiol plus 600 mg testosterone (TE10 group) and 20 mg oestradiol plus 600 mg testosterone (TE20 group). Note cube-root transformed sperm concentration scale. Asterisk indicates significant difference ($P < 0.05$) from control (T) group. Data are expressed as mean and SEM.

Treatment groups differed significantly in semen volume and sperm motility ($P < 0.01$) but without any evidence of a dose-dependent effect of oestradiol. Neither abstinence time (overall mean $2.8 \pm 0.4$ days) nor sperm morphology changed significantly over time or differed between treatment groups.

Hormones

Plasma oestradiol showed a striking dose-dependent peak at the first monthly blood sampling (Figure 2). At 1 month, plasma oestradiol peaked at $192 \pm 23$ pmol/l for TE20, $149 \pm 6$ pmol/l for TE10 and $96 \pm 7$ pmol/l for T groups. These represented an increase of $92 \pm 24$ pmol/l (97 \pm 27\%), $50 \pm 6$ pmol/l (53 \pm 9\%) and $4 \pm 4$ pmol/l (5 \pm 4\%) respectively over their own pretreatment baselines. Subsequently, by the fourth month, there was no longer any significant difference in oestradiol concentrations between oestadiol treated groups; however, both oestradiol treated groups demonstrated an unexpectedly prolonged, continuous low-grade oestradiol release until the end of the study. In contrast, the men who did not undergo oestradiol implantation had stable plasma oestradiol levels throughout the study.

Testosterone concentrations were stable within the middle of the eugonadal reference range throughout the study for the T and TE10 groups whereas the TE20 group demonstrated a transient decrease in total and free testosterone concentrations between months 2 and 6 (Figure 2). Subsequently after the first 6 months, plasma total and free testosterone concentrations did not differ between groups or over time.

Plasma LH concentrations were reduced in all treatment groups with the time-course of the T and TE10 groups being similar whereas the TE20 group had markedly greater and prolonged suppression (Figure 3). Plasma SHBG concentrations were unchanged over time or between treatment groups (Figure 3).

Safety

No unexpected or major adverse clinical effects were reported nor any biochemical abnormalities (including liver function tests) observed. There were few spontaneous reports of minor adverse effects in the T (pellet site pain, one; libido increased, one; libido decreased, one; snoring, one) or TE10 (pellet extrusion, one; pellet site pain, one; nipple tenderness, one) groups compared with the TE20 group (15). In the TE20 group most (11/15) complaints were attributable to symptoms of androgen deficiency ± oestrogenic effects (lethargy and/or tiredness, four; nipple tenderness, two; gynaecomastia, two; libido decreased, three) usually between the fourth and sixth month after entry. The other reports of minor adverse effects in the TE20 group included single reports of pellet extrusion, pellet site pain, increased energy and acne. Twelve men reported no side-effects and three men reported more than one side-effect. Gynaecomastia, reported spontaneously and confirmed by palpation, was present in two men in the higher oestradiol dose group. None of the other participants either reported or had palpable evidence of gynaecomastia.

There were no significant changes in total, high density
lipoprotein or low density lipoprotein cholesterol (Figure 4) or triglycerides (Figure 4) according to treatment group. There were no significant changes in PSA concentrations (Figure 4) or in haematological variables (haemoglobin, haematocrit, leukocyte or platelet counts; data not shown) between treatment groups. There was a small but significant decline in haemoglobin and haematocrit levels equally in all groups over time.

There were no significant changes in body weight, lean body mass, fat mass or percentage fat as measured by bioimpedance analysis according to treatment group or over time (data not shown).

Discussion

The present study demonstrates that oestradiol significantly but modestly enhances testosterone-induced suppression of spermatogenesis in humans, vindicating Ewing’s prediction from extensive animal experiments (Desjardins et al., 1973; Ewing et al., 1973, 1977, 1979, 1983a,b; Berndtson et al., 1975; Lobl et al., 1983). Pioneering studies by Ewing and associates over the decade from 1974 to 1983 demonstrated that the addition of a low dose of oestradiol to testosterone provided optimal suppression of spermatogenesis, superior to that of testosterone alone where sex steroids were administered via a true parenteral steroid depot (Ewing et al., 1983a; Lobl et al., 1983). In their studies using non-biodegradable silastic implants, doses of testosterone and oestradiol approximating the endogenous production rates when combined provided highly effective and reversible suppression of spermatogenesis and fertility (Ewing et al., 1983a; Lobl et al., 1983) with no adverse effects on somatic tissues of rhesus monkeys (Ewing et al., 1983b). Similar results were also obtained in rats and rabbits (Desjardins et al., 1973; Ewing et al., 1973, 1977, 1979; Berndtson et al., 1975) but this concept had never been tested in humans. Despite confirming that oestradiol augments testosterone-induced suppression of human spermatogenesis, the present study also shows a relatively narrow therapeutic margin and, even at maximally tolerated doses of parenteral oestradiol, the suppression of spermatogenesis is still inadequate by the standards required to provide reliable contraception by modern standards (WHO Task Force on Methods for the Regulation of Male Fertility, 1990, 1996). Hence it is unlikely that a depot testosterone–oestradiol combination could form the basis of a practical depot hormonal contraceptive regimen for men.

The present study extends our previous findings using depot steroid implants (Handelsman et al., 1990, 1992, 1996; Handelsman, 1998) in which it was shown that over the full range of testosterone daily release rates covering the normal daily production rates the highest dose of testosterone alone (six 200 mg; 7.8 mg/day testosterone release rate) produced equally effective spermatogenic suppression to that of testosterone enanthate 200 mg per week used in the WHO studies reducing effective weekly testosterone dosage by >50% and eliminating some undesirable metabolic androgen effects, uniform azoospermia could not be achieved with testosterone depot alone in Australian men. On the other hand a suboptimal testosterone dose (800 mg, 5.2 mg/day) when combined with a depot progestin [single i.m. injection 300 mg depot medroxyprogesterone acetate (DMPA)] produced near uniform azoospermia. Similar additive effects of oral progestins with testosterone have been confirmed by other groups (Bebb et al., 1996; Meriggiola et al., 1996, 1997, 1998; Wu, 1999). Nevertheless, regimens combining an oral and injectable medication for male contraception are inherently impractical. A major advantage of a combination depot hormonal approach is not only greater convenience but also avoiding the undesirable metabolic effects inherent in an oral medication due to its inevitable first-pass hepatic overdosage.

The effectiveness of oestradiol in augmenting spermatogenic suppression is illustrated by the dose-dependent enhancement in the decreasing sperm output although the modest magnitude of the oestradiol effect is such that the increased proportions achieving azoospermia were not statistically significant in this study. This discrepancy was due to the loss of statistical power when comparing analysis of sperm output as a continuous variable over time compared with as a dichotomized categorical variable. The dose-dependent increases in plasma oestradiol were also associated with dose-dependent decreases in plasma LH and FSH indicating that the enhanced suppression of spermatogenesis was due to more complete inhibition of pituitary gonadotrophin secretion via steroidal negative feedback. It is difficult to explain the apparent discrepancy between extent of gonadotrophic and spermatogenic suppression accord-

Figure 3. Plot of plasma LH (IU/l, top panel), FSH (IU/l, middle panel) and sex hormone binding globulin (nmol/l, lower panel) reducing effective weekly testosterone dosage by.

Asterisk indicates significant difference (P < 0.05) from control (T) group. Horizontal dashed lines indicate reference range in eugonadal young men. Data are expressed as mean and SEM.
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Figure 4. Plot of plasma cholesterol (mmol/l, upper panel) and triglycerides (mmol/l) and prostate-specific antigen (ng/ml, lower panel) against time in months. The upper panel illustrates total, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol. The lower panel plots triglycerides (TG) against the y-axis depicted on the left axis and PSA against the y-axis depicted on the right axis. Groups of men were as for Figure 1. None of the variables demonstrated any significant difference between groups or over time. Data are expressed as mean and SEM.

Adjusting for baseline sperm concentration (by examining decrement) showed no effect of baseline sperm concentrations on gonadotrophin and spermatogenic suppression; this does not prove, however, that baseline differences were irrelevant. In none of the arms were gonadotrophins fully suppressed which highlights the need for complete gonadotrophin suppression as a necessary, but not sufficient, condition for producing the complete inhibition of sperm output required for reliable male contraception. Although these effects of oestradiol appear to be manifest via central inhibition of gonadotrophin secretion, it is, however, impossible to fully rule out additional direct effects of oestradiol on testicular function. The limitations of oestradiol illustrated in the present study were that while the lower dose had negligible effects, only a two-fold higher dose produced dose-limiting adverse effects. Hence oestradiol has a narrow therapeutic window and, even at higher doses that would not be clinically acceptable, the proportions achieving azoospermia, however, was far from sufficient. It is unclear whether the pattern of oestradiol effects would also apply to the newer mixed partial synthetic oestrogen agonists such as tibolone and raloxifene which interact with the same oestrogen receptors.

An additional implication arising from the present study for the further development of male hormonal contraception is that, for novel synthetic androgens, aromatizability is a desirable property for efficacy although its risk:benefit ratio for safety and acceptability would depend on the quantitative importance and potency of any oestrogenic metabolites arising. For example, the synthetic nandrolone derivative, 7α-methyl, 19-nortestosterone (MENT), being developed for possible application in male contraception (Sundaram et al., 1993), is metabolized to a potent synthetic oestrogen, 7α-methyl oestradiol (LaMorte et al., 1994, 1995). The present study predicts that this active metabolite may enhance spermatogenic suppression but the degree to which it may produce other oestrogenic effects will influence its overall acceptability.

The occurrence of androgen deficiency/oestrogen excess symptoms in the higher oestradiol dosage group appears to be due to both the unexpectedly prolonged duration of action of the oestradiol implants that exceeded the duration of action of the relatively low testosterone implant dosage. The testosterone dose in this study (600 mg) is lower than is currently used for androgen replacement therapy (800 mg) in naturally occurring androgen deficiency states (Handelsman, 1998). Nevertheless our previous pharmacokinetic studies indicated clearly that the 600 mg testosterone dose should be sufficient for 4 months (Handelsman et al., 1990). The unexpectedly prolonged low concentrations of oestradiol released from 20 mg implants lasted many months longer than the expected 2–3 month duration of action in women. Such low levels of oestradiol release would not have been detectable either clinically or biochemically in women but apparently still exert biologically significant effects in healthy eugonadal men. The side-effects consistent with oestrogen excess and/or androgen deficiency were reported between 4 and 6 months after hormone administration. This suggests that, despite plasma testosterone concentrations still within the eugonadal reference range, there was a state of partial androgen deficiency due to unopposed oestradiol release continuing to inhibit endogenous androgen secretion. The fact that higher oestradiol levels (250–
300 pmol/l) sustained for up to 18 months without such side-effects in the WHO studies using weekly i.m. injections of 200 mg testosterone enanthate (Handelsman, personal communication) suggests that the side-effects observed in this study might have been overcome or prevented if exogenous testosterone had been maintained for longer such as with a higher starting dose (Handelsman et al., 1990).

In men a safety concern regarding oestradiol supplementation that did not materialize was the hypothetical risk of arterial thromboembolism. This apprehension originates from the VA-CURG (Veterans Administration Cooperative Urological Research Group) studies in the 1960s (VACURG, 1967) and the Coronary Drug Project in the 1970s (Coronary Drug Project Research Group, 1970; Anonymous, 1973) in which older men being treated with high dose oral oestrogen manifested excess deaths due to arterial thromboses. Recent studies show that parenteral administration of high dose oestradiol, resulting in 10-fold higher circulating oestradiol concentrations than in the present study, has minimal adverse effects on safety (including vascular thrombosis) in older men with prostate cancer (Carlstrom et al., 1997; Henriksson et al., 1999). These findings suggest the absence of arterial thrombosis in this study may reflect predominantly the non-oral route of oestrogen administration rather than the lower oestrogen dosage and the younger age of participants in the present study. The safety experience in this study was also in keeping with the observation that higher oestradiol concentrations (250–300 pmol/l) had been sustained for up to 18 months in men receiving weekly i.m. injections of 200 mg testosterone enanthate (WHO Task Force on Methods for the Regulation of Male Fertility, 1990, 1996) without significant evidence of thromboembolic events (Wu et al., 1996).

The present study again illustrates the advantages of depot hormonal regimens. In addition to the major user-friendly feature of prolonged intervals between drug administrations that is particularly well suited to improving compliance in men, the parenteral route also reduces metabolic side-effects by avoiding the first-pass hepatic overdosage effects which are inherent with the oral route of administration. Future better androgen depots such as testosterone undecanoate (Zhang et al., 1998) or testosterone buciclate (Behre and Nieschlag, 1992) may be developed although these have still relatively shorter duration of action (2–3 months) compared with testosterone implants (4–6 months) as well as large injection volumes leading to discomfort from injections (Mackey et al., 1995) and less predictable pharmacokinetics (Minto et al., 1997). The utility of potent synthetic androgens like MENT or newer synthetic androgens (Avery et al., 1990) as parenteral depots remains to be clarified. The mild asymptomatic fall in haemoglobin in all three groups during the study is hard to explain. There was insufficient blood sampling to account for this being due to venesection, nor was there any evidence of androgen deficiency in the control or lower dose oestradiol groups.

An unexpected finding of note in this study is that, despite randomization, the TE20 group had significantly higher baseline sperm concentrations. This illustrates the fact that randomization, while unquestionably the best guarantor of balance for known as well as unknown covariates at entry into a study, cannot always ensure balance. The use of unbalanced (‘urn’) randomization in this study did allow optimal efficiency in distribution of subjects and final study arm size. The observation that men entering the study later had significantly higher sperm concentrations than those entering early is reminiscent of an earlier observation that recruitment of self-selected volunteers for research studies on sperm output can produce major and unpredictable bias (Handelsman, 1997). In this instance, a similar bias was observed even within the framework of a single study extending the previous observation of such bias between studies within a single centre and the same investigators. The particular susceptibility, of studies requiring semen samples, to participation bias further emphasizes the invalidity of extrapolations of semen findings from uncontrolled, convenience samples of unrepresentative self-selected volunteers (Carlsen et al., 1992). Recruitment to studies requiring semen samples is difficult and various recruitment strategies are utilized. Seasonality seems an unlikely explanation in our study, as recruitment for all arms overlapped seasons. Seasonal effects on human sperm output are, at best, inconsistent and smaller in magnitude than that observed between groups in this study.

The conclusion from this study that a testosterone–oestradiol hormonal combination cannot form the basis of a useful depot regimen for male contraception. Nevertheless aromatization of a potent synthetic androgen is a positive feature for efficacy but may have ambiguous value for safety and acceptability. This study again highlights the advantages of depot hormonal approaches for hormonal contraception for men and further studies of improved depot formulations would be of great interest.

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