

Depot testosterone with etonogestrel implants result in induction of azoospermia in all men for long-term contraception

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BACKGROUND: Combined testosterone and progestogen preparations are a promising approach to male hormonal contraception. We investigated the effect of s.c. etonogestrel with depot testosterone on spermatogenesis in normal men over a period of 48 weeks. **METHODS:** Fifteen healthy men received three s.c. 68 mg etonogestrel implants. Testosterone pellets (400 mg) were administered at 12 weekly intervals. **RESULTS:** Nine men completed 48 weeks of treatment. Four subjects chose to discontinue after 6 months, one man withdrew from the study early for personal reasons and one was withdrawn due to illness. Sperm concentrations of $<1 \times 10^6/\text{ml}$ were achieved in all men by 16 weeks of treatment. All men became azoospermic, although the time to achieve this varied from 8 to 28 weeks. Azoospermia was maintained in eight of the nine men treated for 48 weeks, one subject showing partial recovery from 40 weeks. Testosterone levels remained in the physiological range throughout. Treatment did not result in weight gain, change in body composition or decline in high-density lipoprotein cholesterol concentrations. **CONCLUSIONS:** The combination of three etonogestrel implants with depot testosterone results in rapid and consistent suppression of spermatogenesis. This can be maintained for up to 1 year and may therefore be a suitable approach for a long-acting male hormonal contraceptive.

Key words: etonogestrel/male contraception/progestogen/spermatogenesis/testosterone

Introduction

Current approaches to male hormonal contraception are based on the withdrawal of the gonadotrophin support to the testis resulting in suppression of spermatogenesis and intra-testicular testosterone (Anderson and Baird, 2002; Nieschlag *et al.*, 2003). Two large international studies sponsored by the World Health Organization (WHO) administering high dose testosterone enanthate demonstrated that hormonal suppression of spermatogenesis sufficient for contraceptive efficacy is a possibility (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1990, 1996). However, such regimes resulted in metabolic side effects such as a fall in high-density lipoprotein cholesterol (HDL-C) concentration, acne and weight gain due to supra-physiological testosterone levels (Wu *et al.*, 1996), and revealed ethnic polymorphism in spermatogenic response, with a lesser suppression among Caucasians than in Asians. Progestogens are effective suppressors of gonadotrophin secretion, but the concomitant fall in testosterone production by the testis necessitates the co-administration of androgen. The introduction of a progestogen acting synergistically with testosterone allows a lowering of the total testosterone dose

avoiding unwanted metabolic effects, and may also enhance the degree of spermatogenic suppression, potentially by direct intra-testicular effects (Bebb *et al.*, 1996; Meriggiola *et al.*, 1996; McLachlan *et al.*, 2002; Zhang *et al.*, 2003). Several progestogens have been investigated in this context, including medroxyprogesterone acetate (Knuth *et al.*, 1989; World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1993; Handelsman *et al.*, 1996; Turner *et al.*, 2003), levonorgestrel (Bebb *et al.*, 1996), cyproterone acetate (Meriggiola *et al.*, 1996, 1998) and norethisterone enanthate (Kamischke *et al.*, 2001). Promising results have been obtained using oral desogestrel, with high rates of azoospermia achieved in men from several ethnic backgrounds (Wu *et al.*, 1999; Anawalt *et al.*, 2000; Kinniburgh *et al.*, 2002)

Compared with oral administration, a long-acting drug delivery system has advantages, including dose-sparing and the avoidance of hepatic exposure to high doses, both of which may contribute to the reduction of unwanted adverse effects. Moreover, it may be preferred by some individuals because of ease of compliance (Martin *et al.*, 2000a). Etonogestrel, the active metabolite of oral desogestrel, has

been marketed recently in many countries as a long-acting implant (Implanon[®], NV Organon, Oss, The Netherlands) providing 3 years of contraceptive efficacy in women. We have reported previously our experience with one or two etonogestrel implants in combination with depot testosterone pellets (Anderson *et al.*, 2002). Although profound suppression of spermatogenesis with minimal non-reproductive side effects was induced, azoospermia was achieved in only 64 and 75% of the one and two implant groups, respectively. Etonogestrel implants release $\sim 50 \mu\text{g/day}$, thus even with two implants the daily dose is markedly lower than the optimally effective dose of $300 \mu\text{g}$ desogestrel, which has $\sim 80\%$ oral bioavailability (Hasenack *et al.*, 1986). There was therefore evidence for significant dose-sparing with the implant preparation but, as spermatogenic suppression was not complete in all men, we hypothesized that the addition of a third etonogestrel implant may enhance this spermatogenic suppression. In this study, we additionally have extended the duration of treatment to 48 weeks to investigate whether the steady decline in etonogestrel release from the implants will maintain suppression of gonadotrophins and thus spermatogenesis for that length of time, using the same testosterone regimen we have used previously in the investigation of both oral desogestrel and etonogestrel implants.

Methods

Subjects

Fifteen healthy men (mean age 31.6 years, range 18–37) were recruited from the same general population as previous studies (Anderson *et al.*, 2002; Kinniburgh *et al.*, 2002). Inclusion criteria included age (18–45), mentally and physically healthy, body mass index (BMI) between 18 and 32 kg/m^2 , normal pre-treatment FSH, LH and testosterone concentrations, routine haematological and biochemical analyses, two normal semen analyses according to WHO criteria at least 2 weeks apart, and a normal physical and andrological examination. Pre-treatment sperm concentrations were $> 20 \times 10^6/\text{ml}$ in all men, and motility and morphology were within normal ranges for the local population. Subjects provided written informed consent and the study had ethical approval from Lothian Reproductive Medicine Ethical Review Committee. The study was performed according to GCP guidelines.

Study design and medication

This study was a single-group open investigation of the effects of etonogestrel implants with testosterone pellets. The duration of the treatment period was 48 weeks, with those subjects who were not azoospermic discontinuing treatment if they wished at 24 weeks. Following pre-treatment assessment, three implants each containing 68 mg etonogestrel (Implanon, NV Organon, Oss, The Netherlands) were inserted s.c. in the medial aspect of the non-dominant upper arm to all subjects. All subjects additionally received 400 mg testosterone pellets ($2 \times 200 \text{ mg}$, NV Organon) inserted s.c. under local anaesthetic into the anterior abdominal wall on the day of insertion of the etonogestrel implants, and 12 weekly thereafter for the duration of the treatment period, i.e. at 12, 24 and 36 weeks.

During treatment and recovery, subjects attended at 4 weekly intervals for medical review, and for semen analysis and venesection. Additional blood samples were drawn pre-treatment and at weeks 4 and 12 between 07.30 and 09.30 (a.m. samples) and

between 16.30 and 18.30 (p.m. samples) for testosterone measurement. Subjects were examined at weeks 12, 24, 36, 48 and at final visits, and a morning first-void urine sample was obtained at the same time points for measurement of epitestosterone. Bio-electrical impedance was determined as described (Davies and Preece, 1988; Gregory *et al.*, 1991) using the Holtain Body Composition Analyser (Holtain Ltd, Dyfed, UK) and fat-free mass and percentage body fat determined for each subject at screening, 12 weekly thereafter and at 16 weeks of the recovery period. Throughout the study, any adverse events were noted at each visit. During the recovery phase, subjects attended at 4 weekly periods for a minimum of 16 weeks up to 24 weeks until semen analysis returned to normal by WHO criteria. Subjects with semen analysis below normal WHO criteria were followed-up beyond this period until normal values were attained.

Assays

Blood samples were obtained in fasting subjects (for glucose and lipids) and plasma separated by centrifugation at $4000 g$ for 15 min and stored at -20°C until hormone assay. Testosterone was measured by radioimmunoassay (Corker and Davidson, 1978), and LH, FSH and sex hormone-binding globulin (SHBG) by a time-resolved immunofluorometric in-house assay. Assay sensitivity was 0.3 nmol/l for testosterone, 0.5 nmol/l for SHBG, 0.03 IU/l for FSH and 0.15 IU/l for LH. The intra-assay coefficients of variation (CVs) were $< 10\%$ for testosterone, FSH and LH, and 4% for SHBG. The inter-assay CVs were 12.4% for testosterone, $< 10\%$ for FSH and LH, and 8.8% for SHBG. Free testosterone was calculated as described (Vermeulen *et al.*, 1999). Urinary epitestosterone concentrations (aglycone plus free fraction) were determined by gas chromatography–mass spectrometry as described and validated previously (Kicman *et al.*, 1995; Coutts *et al.*, 1997). Between-assay precision was $< 8\%$ for epitestosterone concentrations between 27 and 133 nmol/l , and 13.4% at 5 nmol/l . The assay sensitivity was 0.87 nmol/l . Inhibin B was measured in both serum and seminal plasma by methods previously described (Groome *et al.*, 1996; Anderson *et al.*, 1998) with an assay sensitivity of 7.8 pg/ml . Etonogestrel was measured by in-house radioimmunoassay by Organon NV, assay sensitivity 30 pg/ml . Intra-assay CV was 9% and inter assay CV was 14% . Samples were analysed for general haematological and biochemical values (including total cholesterol and HDL-C) by routine autoanalyser at 12 weekly intervals.

Semen analysis

At all assessments, semen analysis was carried out using WHO methodology (World Health Organization, 1999). Local normal values for motility are $> 27\%$ grade a + b, or $> 36\%$ grade a + b + c and normal morphology $> 15\%$. Azoospermia was confirmed following centrifugation of the whole semen sample. Centrifugation was performed at $3660 g$ for 15 min, and a sample was classified as azoospermic only after a systematic examination of the re-suspended precipitate indicated the complete absence of spermatozoa.

Behavioural assessment

Sexual activity and interest were investigated by means of a structured questionnaire used to quantify sexual activity over the preceding 2 week period (Anderson *et al.*, 1992). This was carried out before treatment and at 12 weekly intervals thereafter.

Statistical analysis

Results are presented as mean \pm SEM. Hormone data were log transformed and semen concentrations cube root transformed before

analysis by ANOVA (analysis of variance) for repeated measures. Paired *t*-tests were used to investigate at what time points significant treatment effects were evident, with the exception of behavioural data which were analysed using the Wilcoxon matched pair test for non-parametric testing. For all comparisons, a *P*-value of <0.05 was considered significant.

Results

Subjects, adverse events and withdrawals

Of the 15 men entering the study, nine completed 48 weeks of treatment. Four chose to leave the study after 24 weeks for personal reasons. One man was withdrawn from the study at 24 weeks due to inter-current illness (acute alcohol toxicity). One man withdrew from the study for personal reasons after 4 weeks treatment; thus data from this individual are not included in the analysis. Adverse events experienced included low mood (three subjects) and testosterone pellet extrusion (two subjects, replacement pellets administered), but none resulted in any subject withdrawing from the study. Removal of etonogestrel implants was uncomplicated in all men. Pre-treatment data are presented in Table I.

Sperm concentrations

There was a profound suppression of spermatogenesis during the study (Figure 1), and all 14 men became azoospermic eventually. After 16 weeks of treatment, sperm concentration in all subjects was below the threshold of $1 \times 10^6/\text{ml}$, with 10 of 14 subjects (71%) azoospermic (Figure 1b). At 24 weeks, 11 men were azoospermic, and sperm concentrations were $<0.1 \times 10^6/\text{ml}$ in the other three. These three were among the nine subjects who continued the study for the full 48 weeks, and all were azoospermic at 28 weeks. The range of time to azoospermia was 8–28 weeks, median 16 weeks. Eight men remained azoospermic until the end of the 48 week treatment period. One man showed partial recovery of spermatogenesis, with spermatozoa detectable at week 40 ($0.7 \times 10^6/\text{ml}$) and sperm concentration increasing to $7 \times 10^6/\text{ml}$ at 48 weeks.

During the recovery phase, 60% of subjects had reached sperm concentrations in the normal range by week 16, and 79% by week 24. Incomplete follow-up data were obtained in the subject who was discontinued from the study due to inter-current illness and in one other man. The remaining two subjects were followed-up until normal sperm concentrations were demonstrated at 32 and 48 weeks after implant removal.

Table I. Pre-treatment values for subjects included in the study

	<i>n</i> = 15
Age (years)	31.6 ± 1.3 (range 18–38)
BMI (kg/m ²)	25.8 ± 0.9 (range 20.5–31.9)
LH (IU/l)	4.0 ± 0.4
FSH (IU/l)	5.3 ± 0.8
Testosterone (nmol/litre)	22.1 ± 1.7

Data are presented as the mean ± SEM.

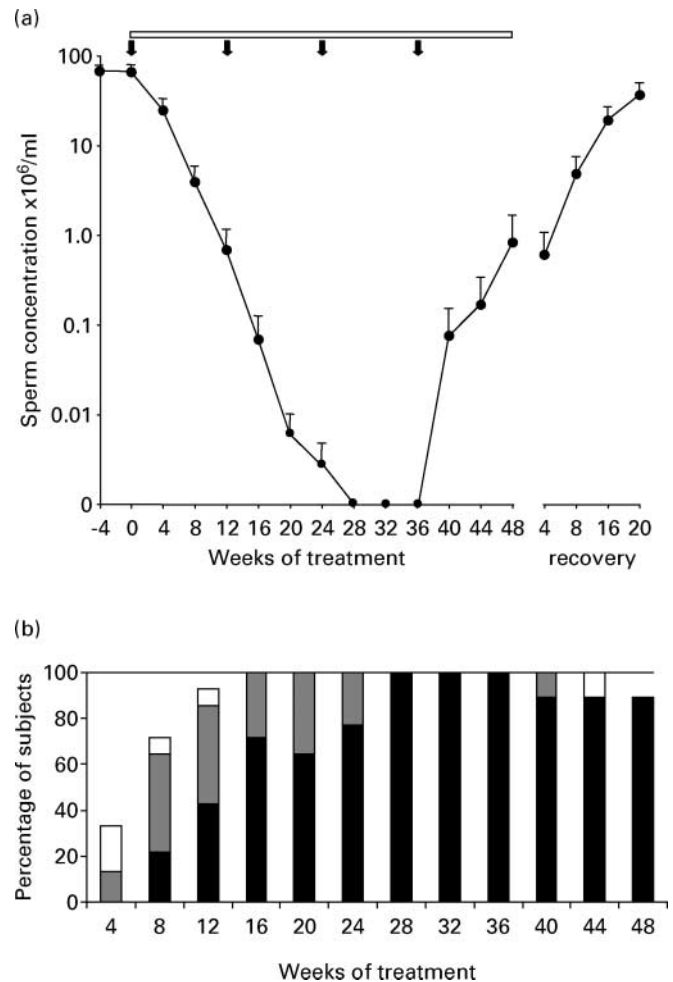


Figure 1. (a) Sperm concentrations during etonogestrel/testosterone treatment and the recovery period. Duration of treatment is indicated by the bars and the time points of testosterone implant insertion are indicated by arrows. Note the log scale on the ordinate. Data are presented as mean ± SEM, *n* = 14 for the first 24 weeks; thereafter nine men continued for 48 weeks. (b) Percentage of men achieving azoospermia (■), and concentrations of $<1 \times 10^6/\text{ml}$ (■) and $1 < 3 \times 10^6/\text{ml}$ (□) at each time point during treatment.

Testosterone and epitestosterone concentrations

Serum testosterone concentrations remained within the normal physiological range throughout the treatment period, with fluctuations according to the timing of testosterone pellet re-administration (Figure 2a). A gradual decline was observed from pre-treatment values reaching statistical significance at week 4 ($P = 0.0006$) with a nadir at week 12. Following re-administration of testosterone at week 12, concentrations rose to levels that were not significantly different from baseline at week 16, with a similar pattern of fluctuation throughout the remainder of the treatment period. During the recovery phase, testosterone concentrations rapidly returned to pre-treatment concentrations. Calculated free testosterone concentrations showed a similar pattern, with nadir concentrations significantly lower than pre-treatment ($P < 0.01$, Table II) and returning to pre-treatment levels during the recovery phase. During the treatment phase, free testosterone concentrations showed a gradual

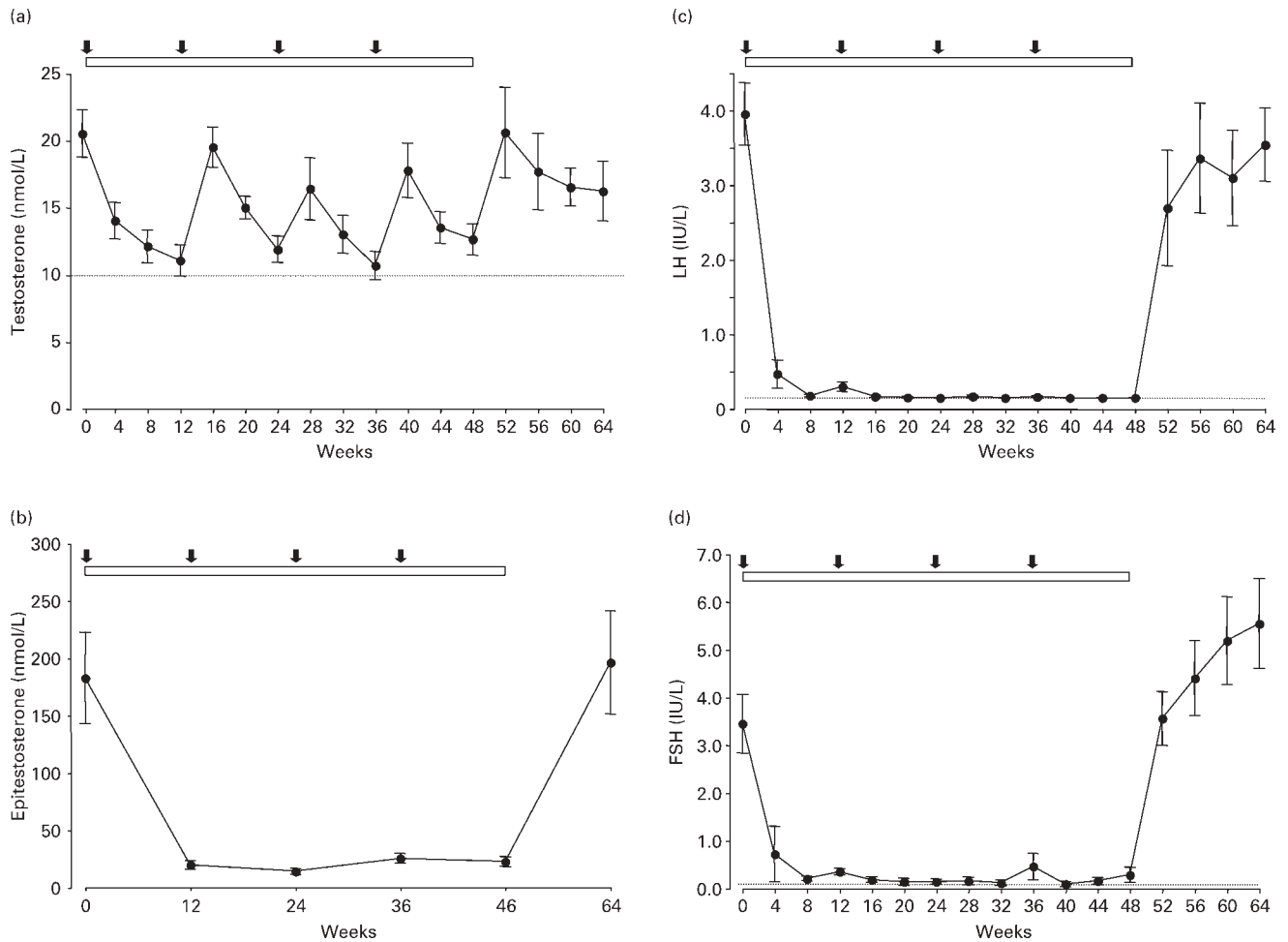


Figure 2. (a) Testosterone, (b) epitestosterone, (c) LH and (d) FSH concentrations during etonogestrel and testosterone treatment. The treatment period is indicated by the bars, with time points of testosterone pellet insertion indicated by arrows. Data are presented as the mean \pm SEM. In (a), the broken line indicates the lower limit of the normal range. In (c and d), the lower limits of detection of the assays are indicated by broken lines; in (b), the limit of detection is 0.89 nmol/l.

rise from week 12 (0.30 ± 0.03 nmol/l) to week 48 (0.39 ± 0.03 nmol/l), which was not statistically significant.

Urinary epitestosterone concentrations were suppressed by week 12 ($P = 0.001$) to $\sim 10\%$ of pre-treatment concentrations (Figure 2b), remaining readily detectable in all samples. Epitestosterone concentrations remained consistently

suppressed throughout treatment without significant change, returning to pre-treatment concentrations by 12 weeks of recovery.

A diurnal variation in serum testosterone concentrations was observed pre-treatment (Figure 3), concentrations in the morning being an average of 35% higher than in the early

Table II. Haematological, lipid, SHBG and free testosterone concentrations pre-treatment and during treatment at the indicated time-points and after 16 weeks recovery

	Pre-treatment	12 weeks	24 weeks	36 weeks	48 weeks	Recovery
Haemoglobin (g/l)	152 ± 1.7	152 ± 2.6	154 ± 1.5	154 ± 2.4	$155 \pm 2.1^*$	$157 \pm 2.1^*$
Haematocrit	0.45 ± 0.01	0.44 ± 0.01	0.45 ± 0.01	0.45 ± 0.01	0.45 ± 0.01	0.46 ± 0.01
Cholesterol (mmol/l) ^a	5.3 ± 0.4	5.0 ± 0.4	$4.6 \pm 0.3^*$	4.9 ± 0.3	4.5 ± 0.3	5.3 ± 0.7
HDL-C (mmol/l)	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.2
LDL-C (mmol/l)	3.7 ± 0.4	3.5 ± 0.4	3.2 ± 0.3	3.5 ± 0.3	3.3 ± 0.2	3.6 ± 0.4
Triglycerides (nmol/l) ^b	2.1 ± 0.3	1.7 ± 0.4	$1.6 \pm 0.2^*$	1.5 ± 0.3	$1.2 \pm 0.2^*$	1.9 ± 0.4
Free testosterone (nmol/l) ^c	0.52 ± 0.05	$0.30 \pm 0.03^*$	$0.34 \pm 0.02^*$	$0.33 \pm 0.03^*$	0.39 ± 0.03	0.44 ± 0.09
SHBG (nmol/l) ^d	25.6 ± 2.7	$18.2 \pm 1.6^*$	$15.5 \pm 1.4^*$	$12.2 \pm 1.9^*$	$13.2 \pm 2.2^*$	22.2 ± 2.9

Data are presented as the mean \pm SEM.

^aCholesterol concentrations were significantly lower at 24 weeks of treatment ($P = 0.006$).

^bTriglyceride concentration was significantly lower at week 24 ($P = 0.05$) and week 48 ($P = 0.02$) of treatment.

^cFree testosterone concentrations were significantly lower from week 12 ($P = 0.003$) until week 36.

^dSHBG concentrations were significantly lower from week 12 ($P = 0.001$) and remained significantly decreased until recovery.

*Further significant ($P < 0.05$) treatment changes following ANOVA.

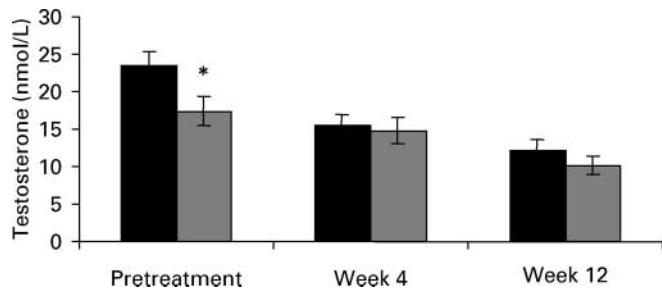


Figure 3. Mean a.m. ■ and p.m. ■ serum testosterone concentrations (nmol/l) in subjects ($n = 11$) receiving etonogestrel and testosterone treatment at pre-treatment and after 4 and 12 weeks treatment. Data are presented as mean \pm SEM. * $P < 0.05$ versus the a.m. sample.

evening ($P = 0.002$). After 4 weeks of treatment, this was lost, with no significant differences between morning and evening concentrations. Concentrations at both times of day at 4 weeks, however, were not significantly different from pre-treatment early evening concentrations. At 12 weeks of treatment, mean testosterone concentrations were low, this being immediately prior to re-administration of the testosterone pellets, but were again similar in the morning and evening. Comparison of the diurnal variation in testosterone concentrations between pre-treatment and 12 weeks showed a significant difference ($P < 0.05$).

Other reproductive hormones

Treatment with etonogestrel and testosterone resulted in profound suppression of both LH and FSH ($P < 0.0001$ versus pre-treatment from week 4 onwards). Some fluctuation in suppression was evident at 12 and 36 weeks (FSH) and 12 weeks (LH), at the times of trough testosterone concentrations (Figure 2c and d). During the later weeks of the study, LH was consistently suppressed to undetectable concentrations in all men at 24 weeks of treatment and for the rest of the treatment period in all men who continued to 48 weeks. Suppression of FSH was more variable, being detectable in up to two-thirds of subjects at time points of trough testosterone concentrations. More consistent partial recovery of FSH concentrations was seen in three men during the final 8 weeks of the study, particularly in the one individual who showed some restoration of spermatogenesis. In this individual, FSH during the second half of the treatment period was undetectable only at week 40, with a mean concentration between weeks 28 and 48 of 0.5 IU/l. Two further individuals with partial escape of FSH suppression (mean concentrations between weeks 28 and 48 of 0.1 and 0.8 IU/l) maintained azoospermia. Both gonadotrophins rapidly recovered following treatment. There was a progressive rise in FSH from weeks 4 to 16 of the recovery phase, at which time FSH concentrations were significantly higher than pre-treatment ($P = 0.02$).

Serum inhibin B concentrations showed a gradual decline over the course of treatment, continuing to week 48 ($P < 0.001$; Figure 4). This reached statistical significance from week 4 of treatment onwards ($P = 0.047$). By week 16

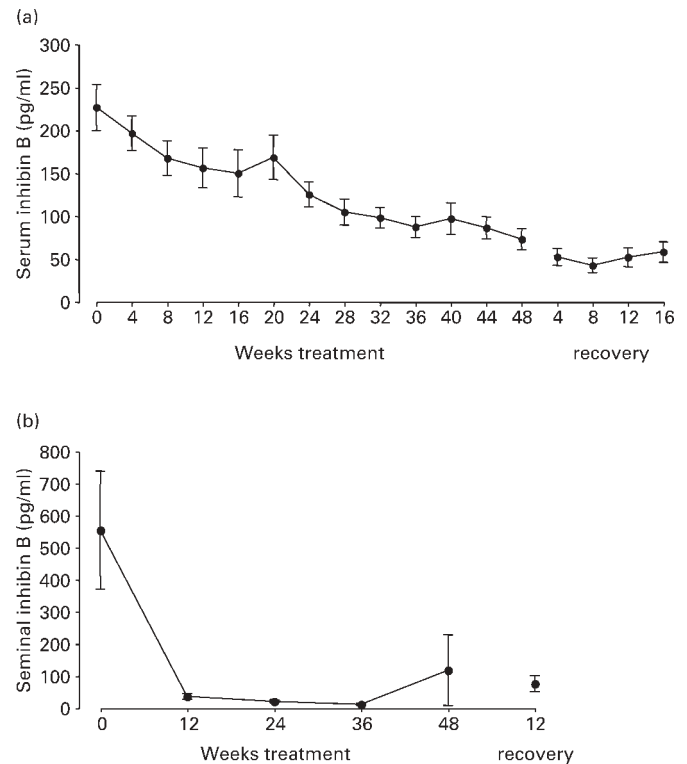


Figure 4. (a) Serum inhibin B and (b) seminal plasma inhibin B concentrations during etonogestrel and testosterone treatment. The treatment period is indicated by the bars, with the time points of testosterone implant insertion indicated by arrows. Data are presented as mean \pm SEM. The large error bar at 48 weeks (b) reflects partial recovery in one individual: seminal plasma inhibin B was undetectable in all other samples at that time.

of the recovery phase, serum inhibin B levels showed only limited evidence of recovery, remaining significantly lower than pre-treatment ($P < 0.001$).

Seminal plasma inhibin B concentrations were profoundly suppressed during treatment ($P = 0.02$ pre-treatment versus week 12). Seminal plasma inhibin B was undetectable in eight of 13 subjects by week 24. In the latter 24 weeks of the study, it was undetectable in all subjects except the individual who demonstrated recovery of spermatogenesis. This subject showed an increase in seminal plasma inhibin B at 36 weeks (having been at the limit of detection at week 24). This thus preceded detectable spermatogenic recovery, as at that time the subject was azoospermic but had a sperm concentration of 0.7×10^6 /ml 4 weeks later.

SHBG showed a gradual decline over the treatment period (Table II). This reached statistical significance by week 4 ($P = 0.0002$) and continued to week 48. During recovery, SHBG returned to pre-treatment concentrations.

Etonogestrel

Serum etonogestrel concentrations were highest 4 weeks after implant insertion, with a mean concentration of 765 ± 57 pg/ml. Etonogestrel concentrations showed a gradual decline thereafter (Figure 5), being 63% of peak levels at 24 weeks and 43% at week 48. Etonogestrel was undetectable in all subjects 4 weeks after implant removal. The individual

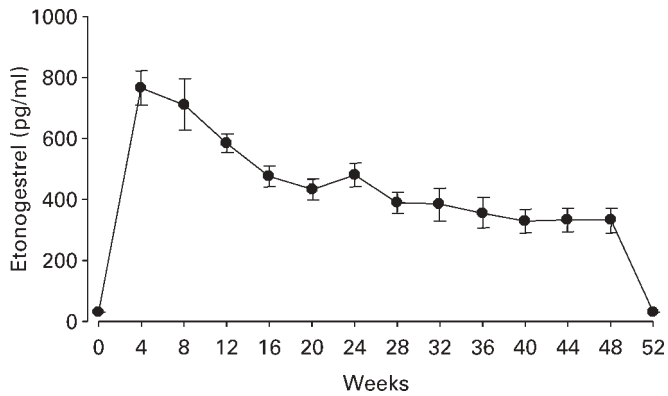


Figure 5. Etonogestrel concentrations during etonogestrel/testosterone treatment (0–48 weeks) and 4 weeks after removal of etonogestrel implants, at which time it was undetectable in all men. Data are presented as mean \pm SEM.

who showed partial spermatogenic recovery had serum etonogestrel concentrations close to the group mean.

Lipids and haematology

Cholesterol concentrations showed a gradual fall during etonogestrel and testosterone treatment, statistically significant at week 24 ($P = 0.006$) and returning to baseline during recovery (Table II). Similarly, there was a decline in triglycerides, reaching significance at week 24 ($P = 0.05$) and week 48 ($P = 0.02$). There was no significant change in HDL-C levels with a small (10%) non-significant decline in LDL-C during the treatment period. There were no significant changes in other biochemical variables during the study period.

A small rise in haemoglobin concentrations was evident at week 48 ($P = 0.003$), which remained elevated during the recovery period. Haematocrit remained unchanged.

Body composition

There were no significant changes in body weight during the treatment or recovery periods. Likewise, body composition analysis showed no changes in fat free mass or percentage body fat (Table III).

Table III. Body composition data (weight, fat-free mass and % body fat) pre-treatment, 12 weekly during treatment and after 16 weeks of follow up

	Pre-treatment	12 weeks	24 weeks	36 weeks	48 weeks	Recovery
Weight (kg)	82.4 \pm 3.4	81.6 \pm 3.5	81.3 \pm 3.2	82.4 \pm 2.8	83.7 \pm 2.9	80.9 \pm 3.5
Fat-free mass (kg)	64.6 \pm 2.2	62.3 \pm 2.4	64.4 \pm 2.6	64.4 \pm 2.6	63.7 \pm 1.9	63.6 \pm 2.4
% Body fat	20.7 \pm 2.3	21.5 \pm 2.6	22.1 \pm 1.9	21.6 \pm 2.8	23.3 \pm 2.9	19.4 \pm 2.5

Data are presented as the mean \pm SEM. No significant changes indicated.

Table IV. Sexual behaviour pre-treatment, 12 weekly during etonogestrel/testosterone treatment and after 16 weeks of follow-up

	Pre-treatment	12 weeks	24 weeks	36 weeks	48 weeks	Recovery
Sexual behaviour	6.2 \pm 1.2	7.4 \pm 1.7*	6.5 \pm 1.8	5.3 \pm 0.8	5.3 \pm 1.5	4.8 \pm 0.9

Data are presented as the mean \pm SEM.

Sexual activity was assessed as the sum of acts of masturbation and intercourse during the preceding 2 weeks.

*A significant increase at week 12 of treatment ($P = 0.04$).

Sexual behaviour

There was a slight increase in sexual activity (recorded as the sum of number of acts of sexual intercourse and masturbation over the preceding 2 weeks) at week 12 of treatment ($P = 0.04$). No changes in sexual activity at other time points were observed during the study (Table IV).

Discussion

One of the major hurdles in the development of a hormonal male contraceptive is the need for sufficient and universal suppression of spermatogenesis. Caucasian populations have shown heterogeneous responses to both testosterone alone and testosterone in combination with progestogens (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1990; Anderson and Baird, 2002), although the addition of a progestogen has generally increased the proportion of men achieving azoospermia. We and others previously have demonstrated very high rates of azoospermia using oral desogestrel as the progestogen (Wu *et al.*, 1999; Anawalt *et al.*, 2000; Kinniburgh *et al.*, 2002). Administration of an implant preparation of etonogestrel, the active metabolite of desogestrel, also resulted in effective suppression of spermatogenesis (Anderson *et al.*, 2002). In the present study, we have explored further both the dose-response relationship and the duration of action of etonogestrel implants when administered with a depot testosterone preparation.

The present data demonstrate profound suppression of spermatogenesis with the combination of three etonogestrel implants and depot testosterone pellets, with all subjects achieving azoospermia. This compares favourably with our previous data using one (64% azoospermia) and two implants (75% azoospermia) over a 24 week period (Anderson *et al.*, 2002) and is similar to that achieved with an oral dose of 300 μ g desogestrel with the same regimen of testosterone administration (Kinniburgh *et al.*, 2002). Although sample sizes do not allow demonstration of statistically greater spermatogenic suppression with three than two etonogestrel implants, increased efficacy is supported by the more

consistent suppression of gonadotrophins and of both serum and seminal inhibin B with three implants. The onset of suppression was rapid, with all subjects having sperm concentrations of $<1 \times 10^6$ /ml by week 16 of treatment. However, the time taken to reach azoospermia was considerably more variable, with three men maintaining very low but detectable numbers of sperm in the ejaculate to up to 28 weeks. Similar data are evident from the recent Australian efficacy study (Turner *et al.*, 2003) despite the very rapid suppression achieved by that combination of testosterone pellets and depot medroxyprogesterone acetate (DMPA), whereby 94% of men achieved a sperm concentration of $<1 \times 10^6$ /ml within 3 months. This may have significant implications for the practicality of the method, depending on the threshold required for acceptable contraceptive efficacy (Nieschlag, 2002).

Serum etonogestrel concentrations of ~ 1200 and 500 – 800 pg/ml were reported for 300 and 150 μ g oral desogestrel, respectively (Wu *et al.*, 1999; Anawalt *et al.*, 2000). In the present study, the serum etonogestrel concentration at 12 weeks was ~ 600 pg/ml. Thus the suppressive effect of this preparation is similar to that of 300 μ g desogestrel per day, whereas the dose is similar to 150 μ g/day. Dose-sparing is also evident with this preparation of testosterone (Handelsman *et al.*, 1992), which maintains relatively stable serum concentrations and particularly avoids the supraphysiological peaks observed with esters such as testosterone enanthate (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1990). The dose of testosterone administered here has no significant suppressive effect on spermatogenesis when given alone (Handelsman *et al.*, 2000), and in combination with a progestogen may be the minimum effective dose. The advantageous features of this testosterone preparation will contribute to minimizing the intratesticular testosterone concentration which is recognized to be of importance in maximizing spermatogenic suppression (Meriggiola *et al.*, 2002; Zhang *et al.*, 2003).

The diurnal variation of testosterone concentrations in adult men has been well characterized (Faiman and Winter, 1971; Bremner *et al.*, 1983) if not understood. The dose of testosterone which is physiological is usually considered to be that which reproduces the peak concentration observed in men during the morning (Nieschlag *et al.*, 1992). This may result in the administration of a higher dose than that required for physiological replacement. In this study, we carried out a preliminary investigation of diurnal variation in serum testosterone before and during testosterone/progestogen administration, which we hypothesized would not be detectable during exogenous steroid administration if it was primarily due to variation in testosterone production rather than metabolism (Southren *et al.*, 1967). The data confirmed that the diurnal variation of testosterone was lost during treatment, at both 4 and 12 weeks. Testosterone concentrations at 4 weeks were similar to pre-treatment evening samples; however, they are probably lower than average over the duration of treatment. While the regimen used here provides the standard replacement dose for hypogonadal men (800 mg every 6 months; Behre *et al.*, 2004), administration of half the total

dose every 12 weeks will result in slight under-replacement over the initial 12 weeks, with steady state reached after the second administration. The average testosterone concentration following second administration was 15.5 nmol/l, which matches accurately the average 24 h concentration determined by frequent sampling in a group of young healthy men (Plymate *et al.*, 1989). This regimen may therefore closely replace testosterone production based on physiological diurnal production rather than morning peaks. The lack of changes in non-reproductive functions such as lipoproteins, haematocrit and body composition observed in this study is strong evidence that the dose administered here (~ 5 mg/day at steady state) provides close to physiological replacement, but this will need confirmation in longer studies assessing a wide range of androgen-dependent functions.

Gonadotrophin secretion was profoundly suppressed during treatment. This was particularly marked with LH. Suppression of FSH was more variable, but greater than with one or two implants (Anderson *et al.*, 2002). The 12 week testosterone administration regimen also appears more effective at preventing FSH escape than the same total dose administered at 24 week intervals (Turner *et al.*, 2003). Desogestrel and other progestogens may result in greater spermatogenic suppression than achieved by comparable gonadotrophin suppression using testosterone alone (McLachlan *et al.*, 2002), consistent with direct testicular effects on steroidogenesis (Satyaswaroop and Gurdip, 1978; El-Hefwany and Huhtaniemi, 1998; El-Hefwany *et al.*, 2000) or androgen metabolism (Mauvais-Jarvis *et al.*, 1974). In the present study, FSH was incompletely suppressed during weeks 24–48 in three subjects, only one of whom showed spermatogenic recovery. While adequate suppression of FSH is clearly necessary for achievement of azoospermia (Narula *et al.*, 2001; Weinbauer *et al.*, 2001), it appears that there is no clear threshold below which azoospermia can be confidently predicted, and that FSH suppression is only one of a number of potential determining factors for incomplete suppression or escape of spermatogenesis. Consistent with the reproducible suppression of LH, urinary excretion of epitestosterone fell to $\sim 10\%$ of pre-treatment values and remained at that level for the duration of treatment. Epitestosterone (17 α -hydroxyandrost-4-en-3-one) is a natural epimer of testosterone secreted predominantly by the testis (Kicman *et al.*, 1999) which therefore provides a measure of endogenous testicular secretion. Epitestosterone excretion during the present treatment regimen was similar to that previously reported during oral desogestrel/testosterone treatment of normal men (Kinniburgh *et al.*, 2002), and is significantly higher than in hypogonadal men (Kicman *et al.*, 1999). Direct measurement of intratesticular testosterone also indicates low ongoing testosterone production despite near complete LH suppression (McLachlan *et al.*, 2002).

The concentration of inhibin B provides an overall measure of Sertoli cell number and function including spermatogenesis (Anderson and Sharpe, 2000). While it would be expected that effective hormonal contraceptive regimens would result in significant falls in inhibin B concentrations, this has not always proved to be the case (Anawalt *et al.*,

1996; Anderson *et al.*, 1997; Zhengwei *et al.*, 1998; Büchter *et al.*, 1999; Martin *et al.*, 2000b; Kinniburgh *et al.*, 2002). We have not found significant changes in our previous studies with both oral desogestrel and etonogestrel implants despite the high prevalence of azoospermia. It is likely that changes in circulating inhibin B require profound regression of spermatogenesis more consistently throughout the testis than is achieved with some regimens (Anderson and Sharpe, 2000). This is supported by testis biopsy data showing variable degrees of spermatogenic regression between nearby seminiferous tubules despite induction of azoospermia (Zhengwei *et al.*, 1998; McLachlan *et al.*, 2002). It is possible that the fall in serum inhibin B contributed to the less consistent suppression of FSH than LH during the latter months of this study. The fall in inhibin B observed in the present study may reflect a greater consistency of suppression than is reflected purely by the prevalence of azoospermia. This is supported by the striking fall in the concentration of inhibin B in the ejaculate. A more variable fall was found in our previous study with one or two etonogestrel implants (Anderson *et al.*, 2002). In the present study, we confirm and enlarge on this finding that changes in seminal inhibin B are a sensitive window into the seminiferous epithelium, as seminal inhibin B was profoundly suppressed in all men to a median of <10 pg/ml at 24 weeks treatment. This is supported by observations in the individual who demonstrated recovery of spermatogenesis during treatment, as the appearance of sperm in the ejaculate intriguingly was preceded by a partial recovery of seminal plasma inhibin B. Interestingly, both serum and seminal inhibin B showed only limited recovery over 16 weeks, while sperm concentrations had largely returned to normal, indicating complex relationships between these various markers of testicular function. Further investigation is required to establish the time scale for recovery of the endocrine function of the seminiferous epithelium following gonadotrophin suppression.

Other approaches using long-acting preparations have involved implants and depot injections. Levonorgestrel has also been administered in implant formulation (Norplant II[®]), with azoospermia achieved in 35% of subjects when given with transdermal testosterone patches and 93% of subjects in combination with weekly testosterone enanthate (Gao *et al.*, 1999; Gaw Gonzalo *et al.*, 2002). The combination with testosterone implants or long-acting injectable preparations has yet to be investigated. 7 α -Methyl-19-nortestosterone (MENT), a synthetic androgen more potent than testosterone and resistant to 5 α -reduction (Sundaram *et al.*, 1993), has also been developed recently as an implant and a potential long-acting male contraceptive. However, even when up to four implants were used (a dose which resulted in significant effects related to excess androgenicity), 30% of men still had significant numbers of sperm in the ejaculate (von Eckardstein *et al.*, 2003) consistent with the limitations of an androgen-only approach in Caucasian men. Thus, of implant approaches to date, the combination presented in this study exhibits higher levels of spermatogenic suppression with a more favourable side effect profile than any of the others. This beneficial therapeutic ratio is likely to reflect the

pharmacokinetics of both the testosterone and progestogen preparations. Other promising long-term approaches include long-acting injectable testosterone undecanoate alone, achieving high levels of oligozoospermia and azoospermia among Chinese men (Gu *et al.*, 2003), the depot injectable combination of norethisterone enanthate and testosterone undecanoate (Kamischke *et al.* 2002), and DMPA with testosterone pellets (Turner *et al.*, 2003).

In conclusion, the results in this study demonstrate that administration of etonogestrel implants at an appropriate dose together with a long-acting testosterone preparation induces profound and consistent suppression of spermatogenesis that can be maintained for a period of 1 year. Whether this time period could be extended remains to be investigated. The maintenance of testosterone concentrations within the eugonadal range and the dose-sparing effects of the delivery methods involving constant release may contribute to the lack of non-reproductive effects. This approach may be a template as the basis for an acceptable, long-acting, and reversible male hormonal contraceptive.

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