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First human exposure to FSH-CTP in hypogonadotrophic hypogonadal males

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BACKGROUND: This is the first report of human exposure to the novel compound follicle stimulating hormone (FSH)-C-terminal peptide (CTP) 'FSH-CTP' (Org 36286), a long-acting recombinant FSH like substance, consisting of the α-subunit of human FSH and a hybrid β-subunit. The latter is composed of the β-subunit of human FSH and the C-terminus part (CTP) of the β-subunit of human chorionic gonadotrophin (HCG). METHODS: In this phase I, non-blind, multi-centre study, 13 hypogonadotrophic hypogonadal male subjects were enrolled to test the safety of FSH-CTP in terms of antibody formation in humans. Furthermore, the pharmacokinetic profile of this new compound was determined. Subjects were injected four times with 15 μg FSH-CTP with an interval of ~4 weeks between each injection. RESULTS: No drug related (serious) adverse events occurred. No antibodies against FSH-CTP or chinese hamster ovary (CHO)-cell derived proteins were detected and measurement of local tolerance demonstrated that s.c. administration of FSH-CTP is well tolerated and no increase in intensity of injection-site responses was observed after repeated exposure to FSH-CTP. After the first and third injection, FSH-CTP serum concentrations were determined. Overall mean (± SD) C_{max} was 0.426 (± 0.116) ng/ml, mean $t_{\frac{1}{2}}$ and $AUC_{0-\infty}$ were 94.7 (± 26.2) h and 81.5 (± 18.8) ng.h/ml respectively. Compared with recFSH (Puregon®), the half life of FSH-CTP was increased 2–3 times. Following the first and third injection a clear rise in serum inhibin-B concentrations were observed. CONCLUSIONS: The use of FSH-CTP is safe and does not lead to detectable formation of antibodies. Furthermore, the pharmacokinetic and dynamic profile of FSH-CTP may lead to the development of new, more convenient regimens for the treatment of male and female infertility.

Introduction

FSH is a pituitary glycoprotein essential for follicular growth in females and for spermatogenesis in males (Schaison *et al.*, 1993; Out *et al.*, 1999). FSH belongs, together with LH, human chorionic gonadotrophin (HCG), and thyroid-stimulating hormone (TSH), to a family of glycoproteins which are heterodimers of two subunits, α and β (Moyle *et al.*, 1998). The α-subunits of these hormones are identical, whereas the β-subunits are hormone-specific and therefore determine the biological specificity. Furthermore, the β-subunit of HCG is distinct from the others due to an extension of the C-terminus ('C-terminal peptide' or CTP) with four O-linked oligosacharides (Boime and Ben-Menahem, 1999). This extension of the β-subunit of HCG is believed to contribute to the prolonged half-life when comparing HCG and LH (31–56 h for HCG and ~20 min for LH respectively) (Damewood *et al.*, 1989; Saal *et al.*, 1991), whereas HCG and LH have a similar affinity for the LH receptor.

FSH is used for clinical treatment of both female and male infertility. Protocols for the induction of follicular development in women and spermatogenesis in men commonly rely on multiple injections of FSH preparations (Schaison *et al.*, 1993; Out *et al.*, 1999). The availability of longer-acting FSH would thus allow the development of new, more convenient treatment regimens. Site-directed mutagenesis and gene transfer techniques have been used to develop such a novel form of FSH, which was designated FSH-CTP (Org 36286). This potentially long-acting recombinant human FSH contains the α-subunit of human FSH and a hybrid β-subunit, which is composed of the β-subunit of human FSH and the CTP part of the β-subunit of HCG.

Preclinical pharmacokinetic studies in dogs have demon-

*For participant details see Acknowledgements section strated a prolonged half-life (~ two times longer) of FSH-CTP compared to that of normal recombinant human FSH (Org 32489, Puregon®). Furthermore, the in-vivo bioactivity of FSH-CTP, as assessed in the ovarian weight augmentation assay (classical Steelman-Pohley test) (Wang, 1988) was also found to be at least two times higher than the in-vivo bioactivity of Puregon (unpublished observations).

The present study is the first in which FSH-CTP has been administered to humans. The three-dimensional structure of this new compound is foreign to humans, and therefore possible antigenicity of FSH-CTP was of special consideration. To avoid complications raised by formation of cross-reactive antibodies against native FSH or HCG, this study was performed in hypogonadotrophic hypogonadal male subjects.

Materials and methods

Subjects

A total of 13 hypogonadotrophic hypogonadal male subjects, who fulfilled the inclusion criteria, were enrolled in the present study. Main inclusion criteria were: FSH and LH serum concentrations $\leqslant$2 IU/l, age $>$30 and $<$60 years, body mass index (BMI) $\leqslant$32 kg/m^2, good general physical and mental health, able and willing to comply with the protocol, and willing to give written informed consent. Main exclusion criteria comprised a history of any disease affecting the immune response, presence of antibodies at screening directed against anti-FSH-CTP or anti-chinese hamster ovary (CHO) cell antibodies, testicular or prostate pathology, hypophysectomy within a period of 6 months, hyperprolactinaemia, history of oncological treatment, and diabetes mellitus. The use of any concomitant medication was not allowed from 14 days prior to the first FSH-CTP injection up to and including the post-treatment assessments, except for testosterone and, if required, pituitary hormone replacement (other than gonadotrophins), or sporadic use of paracetamol (acetaminophen).

The mean age ($\pm$ SD) of all subjects was 45 ($\pm$ 7) years. Mean height was 176 ($\pm$ 7) cm, and the mean weight was 81.7 ($\pm$ 13.7) kg. The mean BMI was 26.4 ($\pm$ 3.3) kg/m^2. For both LH and FSH the median concentrations were 0.5 IU/l. For eight subjects (61.5%), the cause of hypogonadism was hypothalamus-related and for the other five subjects the cause was pituitary related.

Design of the study

The study was designed as a phase I, non-blind, multi-centre study in which the safety, pharmacokinetics, and pharmacodynamics of FSH-CTP were studied in hypogonadotrophic hypogonadal male subjects. The trial protocol was approved by the Independent Ethics Committee (IEC) of each trial centre.

After written informed consent was obtained, the subject's general medical history including his andrological history and drug history were obtained. The subjects included were fully aware of the fact that antibody formation, resulting from FSH-CTP treatment, could impair future infertility treatment. Before any of the included subjects were enrolled, antibody levels against FSH-CTP and CHO cell-derived proteins were measured to prevent occurrence of secondary antibody responses. Following inclusion in the study, subjects received four single s.c. injections of FSH-CTP (solution for injection: 15 μg FSH-CTP in 0.5 ml, NV Organon, The Netherlands) with an interval of ~4 weeks. Subjects were admitted to the clinic in the morning of each day of injection. Prior to and after each injection, blood was collected for the assessment of anti-FSH-CTP and anti-CHO cell or culture supernatant-derived protein antibodies, the concentration of FSH-CTP in serum, to study pharmacokinetics or serum testosterone, inhibin-B, and oestradiol concentrations to study the pharmacodynamics. The assessment schedule is presented in Table I.

Assessments

Safety and local tolerance

Throughout the entire study, occurrence of (serious) adverse events was monitored. The definition used for an adverse event was: 'any untoward medical occurrence in a subject, who is participating in a clinical trial and has been administered a study drug', the definition for a serious adverse event (SAE) was: 'Any untoward medical occurrence that at any time was life-threatening, required in-patient hospitalisation or prolonged existing hospitalisation, resulted in persistent or significant disability/incapacity, was a congenital anomaly/birth defect, cancer, or an overdose, or resulted in death of the subject'.

Antibody levels against FSH-CTP were assessed before and during the study, using a radio-immunoassay based on the formation of an immune complex between the serum-derived specific antibody and [^{125}I]-labelled FSH-CTP. Due to an absence of human antibodies, murine monocolonal antibodies were used as positive controls.

FSH-CTP was produced by CHO-cells transfected with the genes encoding the α- and β-subunits of human FSH and the CTP-part from human chorion gonadotrophin and purified from CHO cell culture supernatant. Residual CHO cell-derived proteins that are present in minute quantities may cause an immune response in subjects treated for infertility with FSH-CTP. To determine the presence of anti-CHO antibodies in human serum after FSH-CTP administration, CHO proteins were coated on 96-well microplates

Table I. Detailed scheme of hormone assessments

Time (h) after injection of FSH-CTP

Pre-dose	1	4	6	8	10	12	16	24	30	36	48	72	96	144	192	240	288	336
Antibodies[a]	● #																	● #
FSH-CTP	● #	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Inhibin-B	● #	●						●			●	●	●	●	●	●	●	●
E_2 and T	● #																	● #

●Assessments after the first and third injection.
#Assessments after the second and fourth injection.
[a]Antibodies to FSH-CTP and anti-CHO cell protein antibodies were also determined post treatment.
E_2 = oestradiol.
T = testosterone.

and incubated with human serum samples. When anti-CHO antibodies are present in a human serum sample, these antibodies bind to the coated CHO proteins. The bound human anti-CHO antibodies were detected by goat anti-human immunoglobulin coupled to horse radish peroxidase (GaH Ig HRP).

Routine biochemical and haematological parameters as well as urine analysis were assessed at screening, 24 h after each administration of FSH-CTP, and at the post-treatment visit.

As the injection of FSH-CTP was given s.c., the injection site (i.e. the abdominal wall around the umbilicus) was examined. Monitoring of the injection site was performed 1 and 24 h after each FSH-CTP injection by the person who administered the study drug. If possible, the same person administered the study drug and monitored the injection site of a subject throughout the whole study. Redness, itching, swelling, pain, and bruising were scored as either none, mild, moderate, or severe reflecting the maximal intensity as experienced by the subject.

FSH-CTP concentrations

FSH-CTP serum concentrations were analysed using an enzyme immunoassay (EIA) without sample purification. In this assay, an antibody, which recognizes the c1 epitope on hFSH (INN-hFSH, Immunological Diagnostics, Austria) was used to coat the 96 well plates. After incubation with serum samples, bound FSH-CTP was detected colourimetrically using biotin-coupled anti-β-HCG monoclonal antibodies (Organon Teknika, Boxtel, The Netherlands), streptavidine coupled to horse-radish peroxidase, and tetramethylbenzidine (TMB) substrate. This assay was developed and validated by NV Organon (Oss, The Netherlands). Calibration curves for FSH-CTP were constructed using 4-parameter-logistic regression. In each analysis series quality control (QC), consisting of human serum, spiked with known quantities of FSH-CTP was analysed. The coefficient of variation (CV) of duplicate determinations did not exceed 20%. The lower limit of quantitation (LLOQ) was 0.079 ng/ml. Selectivity and specificity of the assay were as follows: selectivity: the results of five blank female and six blank male serum samples were below LLOQ. One female blank serum sample contained 0.100 ng FSH-CTP/ml. Specificity: No significant interference could be detected with related and endogenous compounds (i.e. α-FSH-CTP, β-FSH-CTP, recFSH, HCG, hTSH, and hLH + hFSH). Only α-FSH-CTP showed some cross-reactivity (~0.5%). All spiked FSH-CTP serum concentrations were within 80–120% limits (12.6–19.0 ng/ml) of the nominal concentration (15.8 ng/ml).

Measurement of serum hormone concentrations

Measurement of FSH and LH at screening were performed by the laboratory of each participating centre using their standard methods.

Inhibin B in human serum was denaturated (using sodium dodecyl sulphate, SDS at 100°C), oxidised (hydrogen peroxide), and subsequently analysed using an adapted enzyme immunoassay as previously described (Robertson *et al.*, 1996). The detection limit of this assay was 15.6 pg/ml and the CV for duplicate determinations did not exceed 20%. Testosterone and oestradiol serum concentrations were determined by Analytisch Biochemisch Laboratorium b.v. (Assen, The Netherlands) using a time-resolved fluoroimmuno assay (Autodelfia®; Delfia; Wallac Oy, Turku, Finland). The lower limit of quantitation of oestradiol concentrations in serum was 13.6 pg/ml and for testosterone the detection limit was 0.140 ng/ml. The CV of quality controls was $<5\%$ for both the oestradiol and testosterone assays.

Statistical and analytical methods

The statistical analysis of the pharmacokinetic parameters was carried out using a paired *t*-test on log-transformed parameters and a non-parametric signed-rank test on t_{max}. All analyses were performed using SAS Version 6.08 or higher. For pharmacodynamic parameters (testosterone, oestradiol and inhibin-B) only descriptive statistics are presented.

Results

Subject composition

A total of 13 subjects were treated with FSH-CTP. One subject discontinued the treatment after two injections due to unknown reasons. Twelve subjects were exposed to four injections of 15 μg FSH-CTP (total 60 μg) with an interval ranging from 27–39 days. One subject received only two injections with a 6 weeks interval and subsequently discontinued the study. No concomitant medication known to influence the treatment outcome of subjects was used.

Of the subjects that were included, the hypogonadotrophic hypogonadal status was a result of a hypothalamic dysfunction in eight subjects (five idiopathic hypogonadotrophic hypogonadisms and three Kallmann syndromes) and for five subjects this status was caused by dysfunctioning of the pituitary gland (four pituitary tumours and one traumatic lesion of the pituitary gland).

For the safety analysis, data from all 13 subjects was used whereas for the pharmacodynamics and pharmacokinetics analysis, data from ten subjects was used. Three subjects were excluded from the pharmacodynamics and kinetics analysis due to measurable concentrations of FSH-CTP before the first dose was administered, which was probably caused by non-specific interference of serum proteins (see Materials and methods section for the specificity and selectivity of the assay used). In two out of these three subjects, no dose response after FSH-CTP administration was observed at all, in one subject with a positive read-out at t = 0 h a dose response was observed, which peaked at 30 h post administration.

Safety and local tolerance

The safety analysis was performed on all subjects that received at least one injection with the investigated compound. No safety data were excluded from the analysis. In all samples analysed, no anti-FSH-CTP or anti-CHO antibodies were found.

Three subjects (23.1%) experienced a total of five adverse events (maximum intensity): 'skin disorder' (mild), 'periodontal destruction' (moderate), 'abdominal pain' (mild), and twice 'inflicted injury' (mild), none of which was considered drug-related.

All haematology parameters were within the safety limits and no clinically relevant shifts were reported for haematological and biochemical parameters. No clinically significant findings were found at the general medical examination of the post-treatment assessment.

The number of subjects with local tolerance symptoms during the entire treatment period and after the first and last injection of FSH-CTP is given in Table II. All reported reactions had a mild intensity.

Pharmacokinetics

For two out of ten subjects hardly any or no serum samples at all were obtained after the third injection and therefore these subjects were not included in the analysis. The mean curve shows that serum FSH-CTP concentrations are slightly higher after the third injection than after the first injection (Figure 1).

Overall, the mean (± SD) C_{max} was 0.426 (± 0.116) ng/ml, mean t_{max} was 45.9 (± 18.0) h. Because of irregularly shaped curves, the elimination half-life ($t_{\frac{1}{2}}$) and, consequently, $AUC_{0-\infty}$ could not be estimated for all subjects. For the remaining subjects, the mean $t_{\frac{1}{2}}$ and $AUC_{0-\infty}$ were 94.7 (± 26.2) h and 81.5 (± 18.8) ng.h/ml respectively. Descriptive statistics of the FSH-CTP pharmacokinetic parameters are presented in Table III.

No statistically significant difference between the first and third injection was found for all parameters tested, except for $AUC_{0\text{-tfix}}$ and C_{max}. Back-transformation of the mean differences found in the statistical analysis resulted in point estimates of 0.79 and 0.87 for $AUC_{0\text{-tfix}}$ and C_{max} respectively, which means that $AUC_{0\text{-tfix}}$ and C_{max} were 21 and 13% respectively, lower after the first injection as compared to the third injection. No statistically significant difference between the two injections was observed for the time to reach the maximal concentration.

Table II. Local tolerance symptoms during the treatment period

Overall intensity of symptoms	At 1 h after injection		At 24 h after injection	
None (%)	8 (62)		9 (69)	
Mild (%)	5 (39)		4 (31)	
Moderate/Severe	0		0	
Injection no.	1	2	3	4
Number of subjects with symptoms[a]	4	4	2	1

[a]Injection-site responses could be scored as: redness, itching, swelling, pain, and bruising and were classified as either none, mild, moderate, or severe.

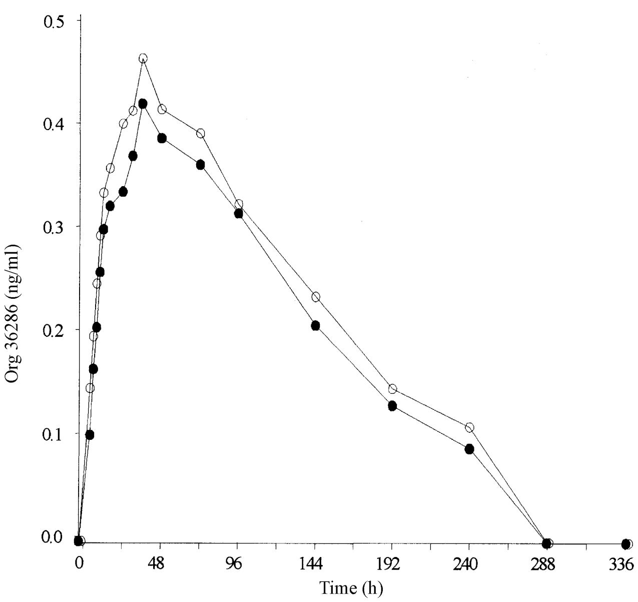

Figure 1. Mean serum FSH-CTP concentration versus time after the first (closed symbols) and third (open symbols) injection (depicted is the average of at least nine concentrations per time point after the first injection and at least seven after the third injection). At t = 0 h, t = 288h and t = 336h concentrations were below the lower limit of quantitation.

Pharmacodynamics

Following the first injection with FSH-CTP, a clear rise in serum inhibin-B concentrations was observed, which returned to baseline concentration before the next injection was given (on average 4 weeks later, Figure 2). Similar effects on inhibin-B serum concentrations were obtained after the third injection with FSH-CTP. The time to reach the maximal concentration of inhibin-B (~144 h) was longer than for FSH-CTP (~46 h). Following administration of FSH-CTP hardly any changes were observed in serum testosterone or oestradiol concentrations. Descriptive statistics on serum testosterone, oestradiol and inhibin-B concentrations are presented in Table IV and Figure 2.

Discussion

This study describes the effects of the first human exposure to FSH-CTP. FSH-CTP is safe and does not lead to detectable formation of antibodies. Furthermore, the pharmacokinetic and dynamic profile of FSH-CTP is very promising for the development of new, more convenient regimens for the treatment of male and female infertility.

The new compound FSH-CTP contains the α-subunit of human FSH and a hybrid β-subunit, which is composed of the β-subunit of human FSH and the C-terminal part of the β-subunit of HCG. This part of HCG contains four O-linked oligosacharides (Feng *et al.*, 1995).

It was hypothesised that the extension of the β-subunit could prolong the half-life of FSH-CTP. In agreement herewith, in the current study, the elimination half-life ($t_{\frac{1}{2}}$) of FSH-CTP in humans was 94.7 ± 26.2 h, which is ~2–3 fold longer than the $t_{\frac{1}{2}}$ of recombinant human FSH. The elimination half-life of recombinant human FSH has been shown to be ~34 h

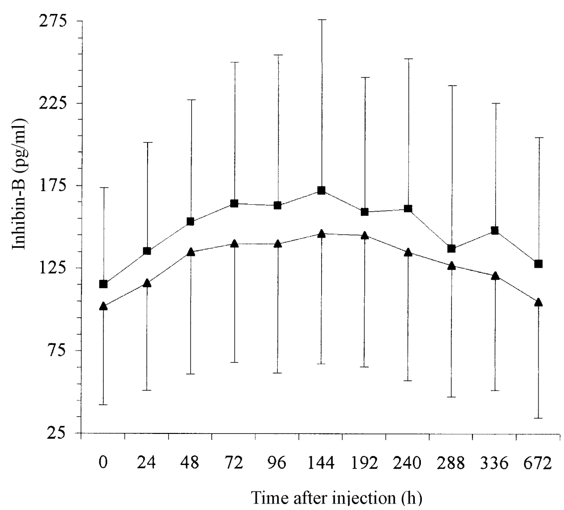

Figure 2. Mean (± SD) serum inhibin-B concentration versus time after the first (triangles) and third (squares) injection (depicted is the average of at least nine concentrations per time point after the first injection and at least six after the third injection).

± 5 h in pituitary suppressed females (33 h ± 4 h using Puregon®Pen) (Voortman *et al.*, 1999). Elimination half-life of recombinant human FSH in a multiple rising dose study in men with hypogonadotrophic hypogonadism was shown to be 48 ± 5 h (Mannaerts *et al.*, 1996).

The three-dimensional structure of this new compound is foreign to humans, and therefore possible antigenicity of FSH-CTP was of special consideration. To avoid complications raised by formation of antibodies against native FSH, this study was performed in hypogonadotrophic hypogonadal male subjects. However, neither anti-FSH-CTP nor anti-CHO antibodies were found in any analysed samples. Furthermore, measurement of local tolerance demonstrated that s.c. administration of FSH-CTP is well tolerated and no increase in intensity of injection-site responses was observed after repeated exposure to FSH-CTP. Finally, no subjects discontinued due to adverse events. Based on these data, it can be concluded that use of FSH-CTP is safe and well tolerated.

Analysis of the mean serum FSH-CTP concentrations after the first injection demonstrated that these concentrations were slightly lower than observed after the third injection. The discrepancy between the first and third injection with FSH-CTP is shown by a statistically significant difference in $AUC_{0\text{-tfix}}$ and C_{max}, albeit that the absolute differences are very small and further research is required to demonstrate the relevance of this observation. When compared with recombinant human FSH, absorption of FSH-CTP appeared to be slow with peak concentrations being reached on average 46 ± 18 h after injection. Recombinant FSH reaches maximal serum concentrations by 13 ± 6 h after s.c. injection in females (Voortman *et al.*, 1999).

To determine pharmacodynamic properties of FSH-CTP, serum testosterone, oestradiol and inhibin-B concentrations were determined. Inhibin-B has been extensively shown to be the physiologically relevant form of inhibin in men (Anawalt *et al.*, 1996). In this study it was shown that the dose of FSH-CTP used caused a rise in inhibin-B concentrations, which returned to baseline concentrations within 4 weeks. This observation is in agreement with recent evidence showing that treatment of oligozoospermic males with FSH increases inhibin-B plasma concentrations (Foresta *et al.*, 1999). Furthermore, the bioactivity of recombinant FSH in hypogonadotrophic hypogonadal male subjects was also reflected by increased inhibin-B levels upon multiple rising doses (Mannaerts *et al.*, 1996). Hardly any changes were observed in serum testosterone or oestradiol concentrations after injection of FSH-CTP. In agreement herewith, serum concentrations of LH, testosterone, androstenedione and oestradiol remained unchanged after administration of increasing doses of recombinant human FSH to male subjects (Mannaerts *et al.*, 1996).

In conclusion, use of FSH-CTP is safe and does not lead to detectable formation of antibodies. This is a promising new compound for development of new, more convenient regimens for the treatment of male and female infertility.

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Table III. Descriptive statistics of the FSH-CTP pharmacokinetic parameters

Injection no.	Statistic	C_{max}(ng/ml)	t_{max}(h)	$t_{\frac{1}{2}}$(h)	$AUC_{0\text{-}\infty}$(ng.h/ml)	Cl_{app}(l/h/kg)
1	*n*	10	10	8	8	8
	mean	0.412	47.7	89.0	76.0	0.00265
	SD	0.120	17.9	18.0	16.0	0.00046
3	*n*	8	8	6	6	6
	mean	0.442	43.6	102.4	88.8	0.00218
	SD	0.117	19.1	34.8	21.2	0.00031
Overall	*n*	18	18	14	14	14
	mean	0.426	45.9	94.7	81.5	0.00245
	SD	0.116	18.0	26.2	18.8	0.00046

C_{max} (ng/ml) = maximal concentration at any time; t_{max} (h) = time at which the maximal concentration is reached; $t_{\frac{1}{2}}$ (h) = Half-life, $AUC_{0\text{-}\infty}$ (ng.h/ml) = area under the concentration-time curve from t = 0 h extrapolated to infinity; Cl_{app} (l/h/kg) = apparent clearance defined by clearance divided by absolute bioavailability.

Table IV. Mean serum testosterone and oestradiol concentrations

		Injection No. 1		Injection No. 2		Injection No. 3		Injection No. 4	
		0 h	336 h	0 h	336 h	0 h	336 h	0 h	336 h
Testosterone (ng/ml)	*n*	10	10	10	10	9	9	9	9
	Mean	36.2	31.8	26.2	29.5	25.0	27.1	24.6	24.9
	SD	26.6	24.3	19.9	20.1	14.0	16.0	18.5	13.2
Oestradiol (ng/ml)	*n*	10	10	10	10	9	9	9	9
	Mean	6.79	6.15	4.84	6.03	6.51	6.31	5.43	5.94
	SD	5.18	4.84	3.33	3.86	4.24	3.65	3.71	4.02

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