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► To cite this version:

Anne Bachelot, Marion Lapoirie, Jerome Dulon, Monique Leban, Raphaele Renard Penna, et al.. Effects of mitotane on testicular adrenal rest tumors in congenital adrenal hyperplasia due to 21-hydroxylase deficiency - a retrospective series of five patients. *European Journal of Endocrinology*, 2021, 10.1530/EJE-20-0787. hal-03121896

HAL Id: hal-03121896

<https://hal.sorbonne-universite.fr/hal-03121896>

Submitted on 26 Jan 2021

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1 Effects of mitotane on testicular adrenal rest tumors in congenital adrenal hyperplasia due to
2 21-hydroxylase deficiency - a retrospective series of five patients

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18 Short title: Mitotane for TART

19 Key words: congenital adrenal hyperplasia, 21-OH deficiency, adrenal rest tumors, fertility,
20 sperm analysis, mitotane

21 Word count: 2359

22

23 ABSTRACT

24 We conducted a retrospective study on the long-term effect of mitotane treatment on testicular
25 adrenal rest tumors (TARTs) in 5 adult patients with classic 21-hydroxylase deficiency,. After
26 60 months of mitotane treatment, a decrease of adrenal steroids was observed in 4 patients.
27 Testicular ultrasonography showed complete disappearance of TART in 2 patients, stabilization
28 in 2 patients and a halving of TART volume in the remaining patient. Sperm count improved
29 notably in 2 patients who had normal baseline inhibin B levels and small inclusions, thus
30 enabling cryopreservation of the subjects' semen. Four years of follow-up of these two patients
31 after the withdrawal of mitotane showed no recurrence of TART and persistent normal
32 testicular function. In conclusion, mitotane could be used as a last resort in CAH patients in the
33 cases of azoospermia associated with TARTs but normal inhibin B levels, as it can improve
34 long-term endocrine and exocrine testicular function.

35

36 INTRODUCTION

37 Twenty-one-hydroxylase deficiency (21OHD) is the most common form of congenital adrenal
38 hyperplasia (CAH). Male patients with CAH may present impaired testicular function and
39 infertility (1). Testicular adrenal rest tumors (TARTs) have been described in these patients (1-
40 4) and have been demonstrated to constitute one of the most important causes of infertility. In
41 recent studies, the overall prevalence of TARTs in classic CAH patients was 40% (1-6). TARTs
42 appear to be associated with poor hormonal control and concomitant elevated
43 adrenocorticotrophic hormone (ACTH) (7,8). Molecular characterization of TARTs has shown
44 that these tumors have multiple steroidogenic properties, including the expression of adrenal
45 cortex and typical Leydig cell markers (9), leading to the hypothesis of pluripotent cells as the
46 origin of TARTs (3). To date, there are no methods to prevent the development of TARTs, nor
47 are there guidelines to treat patients with TARTs (3). Systematic ultrasound evaluation is
48 recommended at puberty to detect lesions at an early stage. A semen analysis should also be
49 done as soon as possible and the question of systematic sperm cryopreservation seems fully
50 justified (3). Treatment options for male patients with TARTs are still limited and mainly based
51 on good hormonal control with glucocorticoids (3,10,11). However, long-term high doses of
52 glucocorticoids do not always restore testicular function and often have unacceptable side
53 effects (3).

54 Mitotane, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chloro-phenyl) ethane, has been used for
55 several decades to treat adrenal carcinoma and Cushing's syndrome based on its potent
56 adrenotoxic effects (12-14) and capacity to block cortisol synthesis by inhibiting 11 β -
57 hydroxylation and cholesterol chain cleavage. Recently, Bry-Gaillard *et al.* have shown that
58 mitotane could restore fertility in CAH patients with TARTs (15). In this study, we suggest that
59 long-term mitotane treatment is able to shrink adrenal rest tumors, improve sperm count and
60 adrenal secretion in 5 adult patients with classical CAH due to 21OHD presenting testicular

61 adrenal rest tumors in whom intensified glucocorticoid therapy was inefficient or impossible
62 due to major side effects.

63

64 **Patients and Methods**

65 *Patients*

66 Medical files from 5 patients were recorded retrospectively. The main clinical features are
67 summarized in Table 1. All patients were diagnosed as newborns with a salt-wasting form of
68 CAH due to 21-hydroxylase deficiency. They were treated with hydrocortisone and
69 mineralocorticoids. Patients 1, 3 and 4 were homozygous for an IVS2 splice mutation, patient
70 2 was compound heterozygous for a large lesion and L307fs and patient 5 was compound
71 heterozygous for R386W and a large lesion. Testicular adrenal rest tumors were diagnosed by
72 testicular ultrasonography in all patients except patient 4, in whom TARTs were diagnosed by
73 clinical examination. Semen analysis showed complete azoospermia in patients 1 and 3 and
74 oligospermia in patients 2 and 5. Semen analysis was not possible in patient 4 (anejaculation).
75 None of these patients had fathered children at baseline. **The 5 patients were treated with
76 glucocorticoids and mineralocorticoids (figure 1) and had already increased their doses of
77 glucocorticoid treatment without any efficacy in reducing TARTs volume. Furthermore, they
78 had side effects of weight gain or osteopenia in response to the glucocorticoid therapy.**

79 Each patient underwent regular follow-up, including routine biochemistry, endocrine work-ups,
80 and physical examinations, to monitor the safety and efficacy of the treatment. The endocrine
81 work-ups included **fasting blood samples** to ascertain levels of measurement of plasma 17OH-
82 progesterone, testosterone (T), androstenedione (A), ACTH, renin, testosterone, sex hormone
83 binding globulin (SHBG), LH, FSH and inhibin B, **in the morning, before medications**. Semen
84 analysis was performed according to the World Health Organization (WHO) guidelines. **Each
85 patient had testicular imaging at 6 months, then at 12 months (except patient 3 and 4), 24 months**

86 (except patient 4), 36 months and 60 months (except patient 2). A testicular sonogram was
87 performed on each patient, except for patient 2 who underwent testicular magnetic resonance
88 imaging (MRI). Treatment compliance was assessed by monitoring plasma mitotane levels
89 every 6 months (14,16).

90 In France, retrospective studies such as ours do not require institutional review
91 board/institutional ethics committee approval to analyze the data and publish the results.

92

93 *Methods*

94 *Mitotane therapy*

95 Mitotane treatment (Lysodren, HRA; 500 mg tablets) was proposed to the 5 patients. It was
96 administered orally at a starting dose of 0.5 to 1 mg daily and then increased to 2 to 3 g daily
97 or the highest tolerated dose. The dose was adjusted by monitoring plasma mitotane levels to
98 maintain drug concentrations under 14 µg/ml, thus avoiding toxicity. The highest doses were
99 0.5 g/d in patient 1, 4 g/d in patient 2, 1 g/d in patient 3, 2.5 g/d patient 4 and 3 g/d in patient 5.
100 Patients 1, 2, 3 and 5 were treated for 5 years. Patient 3 interrupted treatment for approximately
101 18 months following the occurrence of acute adrenal insufficiency. Patient 4 had to stop
102 treatment after only 1 year due to poor digestive tolerance. During treatment, the dose of steroid
103 coverage in the 5 patients was increased to prevent adrenal insufficiency. Discontinuation of
104 treatment was decided according to the response to treatment (improvement or even
105 normalization of sperm analysis, decrease or disappearance of inclusions on ultrasound), or in
106 case of poor tolerance to treatment.

107 *Data analysis*

108 The results are presented as the mean±SD if not stated otherwise.

109

110 **Results**

111 *Adrenal steroids and ACTH*

112 As shown in Figure 1, 17OHP, progesterone and A levels were very high at baseline in
113 4 of the 5 patients. Only patient 3 showed a good hormonal balance under treatment, with
114 17OHP levels at 2.0 ng/ml and A levels at 0.65 ng/ml. **During treatment, the 17OHP and A**
115 **levels of patients 2 remain elevated. After 12 months of treatment, there was a median decrease**
116 **of 88% of serum 17OHP levels and of 57% of serum A levels in the 3 remaining patients (Figure**
117 **1).** Thereafter, hormonal control remained stable during treatment with mitotane, except in
118 patient 2, in whom 17OHP, A and progesterone increased concomitantly with poor compliance
119 with treatment. This was confirmed by the mitotane levels, which remained low during the first
120 two years of treatment (<2 mg/l), (Figure 1).

121

122 *Testicular function and morphological evaluation of TART*

123 The evolution of testicular hormonal function is represented in Table 2. At the initial
124 evaluation, basal serum LH and FSH levels were low in patients 1 and 5, indicating
125 gonadotrophin insufficiency. After 24 months of mitotane treatment, there was a recovery of
126 gonadotropin secretion, and total testosterone levels increased, due to elevated SHBG, which
127 is generally associated with mitotane treatment. Patient 2 had moderate testicular insufficiency
128 after 24 months of treatment. Patients 3 and 4 showed elevated gonadotrophin levels, reflecting
129 testicular failure.

130 The results of testicular investigations are presented in Table 1 and Figure 2. **Each**
131 **patient had testicular imaging at 6 months, showing no change of TARTs volume.** In patients 1
132 and 5, there was a near disappearance of the intratesticular masses by ultrasonography and MRI
133 **at 24 months**, concomitant with the normalization of sperm count. By ultrasonography, there
134 was a relative stability of TART volume during mitotane treatment in patients 3 and 4 and a
135 halving of TART volume in patient 2.

136 The sperm count results before and during mitotane treatment are presented in Table 3.
137 Patients 1 and 5 showed normalized sperm count during treatment, which allowed for the
138 storage of their semen by cryopreservation. Patient 2 had severe oligospermia and azoospermia
139 6 months later. This patient had the largest TART. No sperm analysis was done during the
140 therapy with mitotane in patients 3 and 4, for personal reasons.

141 *Safety*

142 Weight loss was observed in patients 1, 2 and 5, at respectively -8.1%, -15.7% and -
143 18.4%. The weight remained stable for patient 3, and patient 4 gained 9.5 kilos in 6 months
144 (+15.8% under treatment). Patient 4 had to stop the treatment prematurely due to poor digestive
145 tolerance (nausea and diarrhea). **Patients 3 and 5 each experienced one episode of adrenal crisis**
146 **during treatment, respectively after 20 and 5 months of treatment, requiring a short hospital**
147 **stay for each, followed by discontinuation of treatment in patient 3 and a simple adjustment of**
148 **glucocorticoid treatment in patient 5. None of the patients require higher doses of**
149 **fludrocortisone during therapy.** Moderate elevation of LDL-c was observed during treatment,
150 but this symptom resolved with reinforced dietary and lifestyle measures. No patients were
151 treated with statins. Finally, hepatic cytolysis and minimal anicteric cholestasis <1.5N was
152 observed in all patients except patient 2, with complete normalization after discontinuation of
153 the treatment.

154

155 *Long-term follow-up*

156 Long-term follow-up after discontinuation of the treatment was available for patients 1, 4 and
157 5. Two years after the discontinuation of mitotane, patient 1 fathered a healthy child. His last
158 ultrasound 4 years after the discontinuation of treatment found stable testicular volume, with
159 minimal inclusions (0.1 cc on the right, 0.2 cc on the left) and hormonal assays showed normal
160 testicular function (FSH 3.4 IU/l, LH 5.2 IU/l, TT 7 ng/ml, SHBG 60 nmol/l, inhibin B 178

161 ng/ml).

162 One year after discontinuation of mitotane, patient 5 showed a decrease in sperm count,
163 nevertheless allowing for semen cryopreservation. The last ultrasound done 6 years after
164 stopping treatment found testicles of normal volume without TARTs, and normal testicular
165 function (FSH 3.2 IU/l, LH 5.4 IU/l, TT 9.5 ng/ml, SHBG 86 nmol/l, inhibin B 201 ng/ml).

166 Five years after the one year treatment with mitotane, patient 4 still had moderate testicular
167 insufficiency (FSH 13.3 IU/l, LH 9.2 IU/l, TT 6.4 ng/ml, SHBG 38 nmol/l, inhibin B 33 ng/ml)
168 with bilateral testicular hypotrophy by ultrasound (8 cc on the right, 10 cc on the left), and
169 increased inclusion volumes (4 cc on the right, 4.5 cc on the left). Semen analysis five years
170 after stopping treatment showed cryptozoospermia (4 spermatozoa).

171

172

173 Discussion

174 Until now, there have been no clear guidelines for the treatment or prevention of TARTs
175 (3). The development of TARTs is generally understood to be the result of sustained elevation
176 of plasma ACTH concentrations, usually associated with poor control of CAH. However,
177 TARTs have also been reported in men with adequate control of CAH and, conversely, some
178 subjects with poorly controlled CAH never develop TARTs despite chronically elevated ACTH
179 levels. The first choice of treatment is intensification of glucocorticoid treatment to suppress
180 ACTH, based on a few cases in which this has led to reduction of the tumor size and improved
181 testicular function (17-20). Hydrocortisone, dexamethasone, and prednisone were all used as
182 treatment for TARTs (21). However, no prospective studies of TARTs and intensified
183 glucocorticoid treatment have been published.

184 Recently, Bry Gaillard *et al.* reported the case of one CAH patient with TART who was
185 successfully treated with mitotane to restore fertility (15). In our study, we report five patients

186 with TARTs unresponsive to intensified glucocorticoid treatment, which for half of them were
187 successfully treated with mitotane, thus demonstrating the long-term effect of mitotane to
188 improve testicular function and fertility in CAH patients. Two of the five patients presented
189 with hypogonadotropic hypogonadism due to poor hormonal control of CAH. The difficulty in
190 diagnosing hypogonadism in men with CAH is related to the fact that testosterone measured in
191 serum is a mixture of testosterone of gonadal and adrenal origin. Undetectable or low
192 gonadotrophins, associated with high levels of androstenedione and progesterone, should help
193 lead to this diagnostic. Recovery of hypogonadotropic hypogonadism was achieved in these
194 patients under mitotane therapy, thanks to control of the disease. Interestingly, these patients
195 had normal inhibin B levels, suggesting intact Sertoli cells, in accordance with a previous study
196 (10). These patients therefore probably had impaired endocrine and exocrine testicular function
197 due to poor hormonal control and recent TART development. We demonstrate that, in the early
198 stages of TART development, mitotane therapy can lower ACTH levels, reduce TART size and
199 improve testicular function and fertility. Inhibin B may be a good indicator of the success of
200 mitotane therapy.

201 On the contrary, in the later stages of TART development, this approach may be
202 effective at reducing tumor size and improving adrenal function or controlling further growth
203 and testicular damage, but is ineffective at improving testicular function and fertility, due to
204 irreversible damage of the testis. This was the case in patients 2 to 4, who presented testicular
205 failure at baseline or discovered during the mitotane therapy. These patients exhibit low levels
206 of inhibin B at baseline.

207 Mitotane is a metabolite of the pesticide and endocrine disruptor DDT
208 (dichlorodiphenyltrichloroethane) and is classified as a teratogenic compound worldwide
209 (12,13). However, little is known about its effects on human development or its direct effect on
210 oocytes or sperm. A recent study on 4 women having conceived while taking mitotane, or with

211 detectable mitotane plasma levels, showed that exposure of these four children to mitotane in
212 utero seemed to have no clear teratogenic effect (22). **Two studies on reproductive toxicity of**
213 **DDT in adult rats have showed direct testicular impact of these products, with a dose-dependent**
214 **reduction of testicular weight and the number as well as the percentage of motile spermatozoa**
215 **in the epididymis (23,24).** Interestingly, our study shows the absence of TART recurrence and
216 persistence of spermatogenesis in the two responsive patients far from mitotane withdrawal.
217 Therefore, conception is safe at a distance from treatment.
218 However, mitotane does cause irreversible chemical adrenalectomy, and is therefore only
219 recommended in patients who show good compliance to the adrenal replacement therapy.
220 Glucocorticoid doses should be increased during this treatment because it **induces CYP3A4 in**
221 **a major way, leading to more rapid inactivation of hydrocortisone, requiring a 50-100%**
222 **increase in GC dose to avoid adrenal crisis, as experienced by 2 of our 5 patients (25).** None of
223 **our patients experimented hypogonadism symptoms during mitotane treatment, as it has been**
224 **reported in patients with adrenocortical cancer (25).** Finally, liver function and cholesterol
225 metabolism must be closely monitored in these patients (26).

226 In conclusion, patients with CAH should be informed about the risks of the development
227 of TARTs, and consequently the high risk of infertility. They should be monitored regularly by
228 ultrasound and should be offered systematic semen evaluations and cryopreservation. Mitotane
229 should be used as a last resort in men with 21-hydroxylase deficiency and azoospermia in whom
230 azoospermia is associated with large testicular adrenal rest tumors despite normal inhibin B
231 levels, as it can improve endocrine and exocrine testicular function with residual effects lasting
232 several years after withdrawal.

233

234 **Declaration of conflicts of interest**

235 There is no conflict of interest that could be perceived as prejudicing the impartiality of the
236 research reported.

237

238 **Funding**

239 This research was not funded by any specific grant from any funding agency in the public,
240 commercial or non-profit sector.

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- 337
338

339 Figure Legends

340

341 **Figure 1 : Adrenal steroids, ACTH and mitotane levels at baseline and during mitotane**
342 **treatment.**

343 Normal baseline values : 17OH-progesterone (ng/ml) 0.6-3.4; Androstenedione (ng/ml) 0.2-
344 2.9; Progesterone (ng/ml) 0.2-1.4.

345

346 **Figure 2 : 28 Year old man with adrenal hyperplasia who had bilateral testicular masses.**

347 T2 weighted Axial MR (A, B) shows bilateral intratesticular masses that are hypointense
348 compared with normal testicular tissues. Longitudinal sonogram of right testis (C) shows
349 large hypoechoic solid intratesticular masses with sound attenuation, avascular on Color
350 doppler sonogram (D),

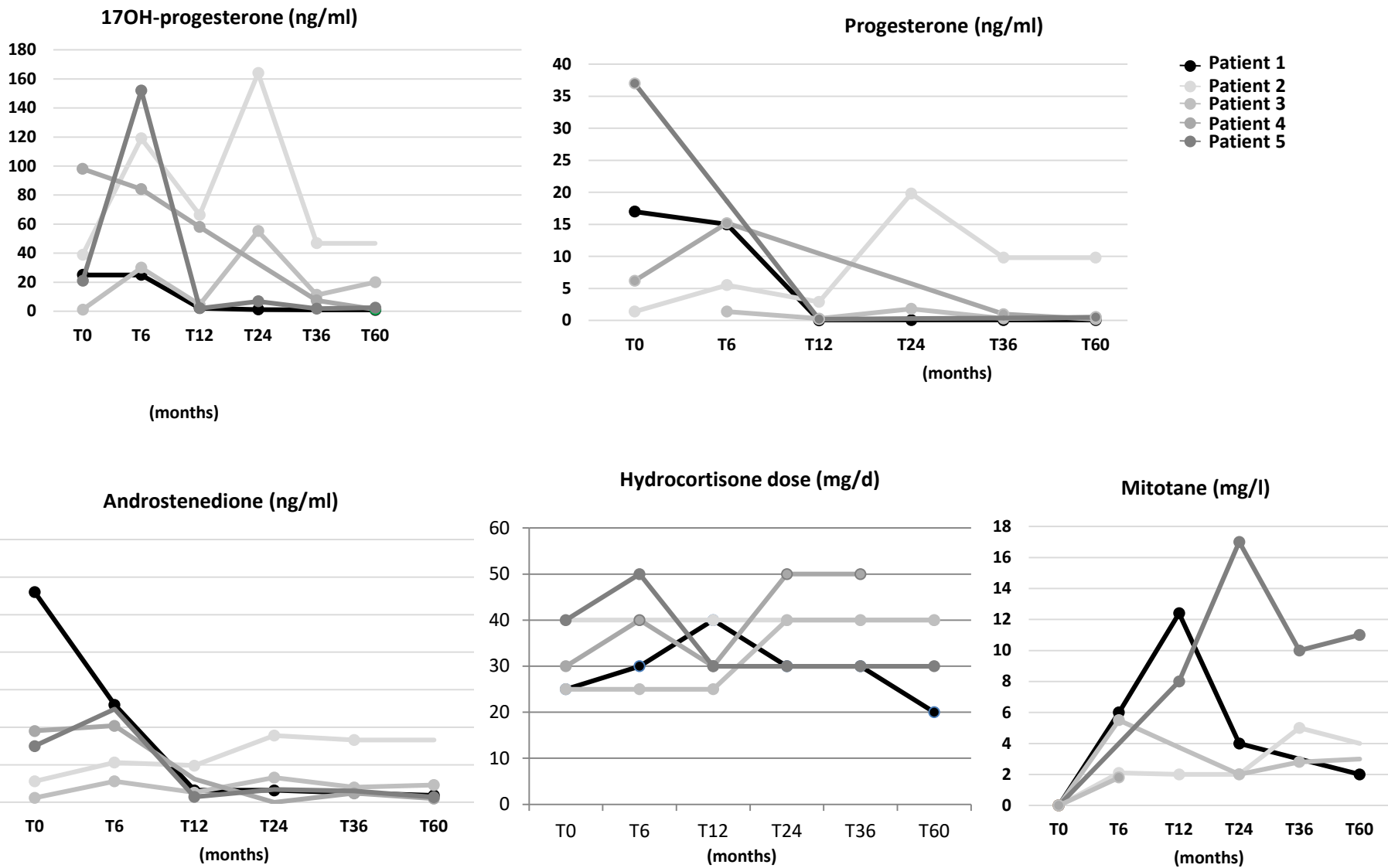


Figure 1 : Adrenal steroids, hydrocortisone dose and mitotane levels at baseline and during mitotane treatment

Normal baseline values :

17OH-progesterone (ng/ml) 0.6-3.4

Androstenedione (ng/ml) 0.2-2.9

Progesterone (ng/ml) 0.2-1.4

Table 2. Evolution of testicular function in CAH patients at baseline and during mitotane treatment

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
FSH (IU/l)					
Baseline	0.1	9.8	17	7.1	1.2
T12	3.3	ND	8.7	4.1	5.2
T24	7.5	8.1	13.1	ND	9.1
T36	6.7	14.4	18.1	15	ND
T60	3.6	ND	14.8	ND	8.2
LH (IU/l)					
Baseline	0.34	9.3	11.2	9.2	1.1
T12	9.1		11.4	4	4.3
T24	14	11.3	12	ND	15.2
T36	11.3	11.6	10.4	ND	9.8
T60	5.4	ND	10	9.8	2.7
Total testosterone (ng/ml)					
Baseline	5.7	8.8	5	6.2	3.4
T12	17.5	2.9	9.8	4.1	9.5
T24	19	7.3	9.3	ND	29
T36	9.3	11.2	11.0	3.7	17
T60	7.5	ND	12.8	ND	8.6
SHBG (nmol/l)					
Baseline	50	43	34	30	32
T12	220	68.4	78	ND	244
T24	128	113	46	ND	172
T36	77	142	61	27	124
T60	70	ND	81	27	87
Inhibin B (ng/ml)					
Baseline	128	131	15	31	167
T12	167	60	71	40	111
T24	ND	46	67	ND	ND
T36	ND	39	43	42	126
T60	152	75	72	33	128

ND: not determined

Normal baseline values:

FSH (IU/l) 1.5-12.4; LH (IU/l) 1.5-8.6; Total testosterone (ng/ml) 2.5-8.4; SHBG (nmol/l) 16.5-55.9; Inhibin B (pg/ml) 25-325

Table 1. Clinical and ultrasonographic characteristics of the 5 CAH patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	24	20	21	28	24
Height (cm)	162	172	172	163	165
BMI (kg/m ²)	28	21	18	23	27
Treatment					
HC	25 mg/d	40 mg/d	25 mg/d	30 mg/d	40 mg/d
FC	100 µg/d	100 µg/d	50 µg/d	150 µg/d	100 µg/d
Testicular volume (left/right, ml)	7/5	18/16	9/11	19/25	12.5/13
TART volume (left/right, ml)					
Baseline	0.8/1	8/8	5/6	4/2.5	0.3/0.2
24 months	0/0	4/2.1	4/6		0/0
36 months	0/0	5/6	5/4.5	2.5/2.7	0/0
60 months	0.1/0.2		4/6.5	2.6/2.1	0/0

HC: hydrocortisone; FC: fludrocortisone; BMI: body mass index; TART: testicular adrenal rest tumors.

Table 3. Evolution of sperm parameters in CAH patients at baseline, during mitotane treatment and after discontinuation of the treatment

Sperm count (x10 ⁶ /ml)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Baseline	azoospermia	1.2 x10 ⁶ /ml	0.06 x10 ⁶ /ml	anejaculation	15 x10 ⁶ /ml
At 12 months	12 x10 ⁶ /ml	azoospermia	ND	ND	azoospermia
Stop of mitotane	24 x10 ⁶ /ml	ND	ND	ND	39 x10 ⁶ /ml
After stop of mitotane (months)	33 x10 ⁶ /ml (16) 15 x10 ⁶ /ml (60)			4/ml (60)	7.2 x10 ⁶ /ml (12)