

## CLINICAL STUDY

**Fertility, sexuality and testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia**

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**Abstract**

**Objective:** Fertility in males with congenital adrenal hyperplasia (CAH) is reported from normal to severely impaired. Therefore, we investigated fertility/fecundity, social/sexual situation, and pituitary-gonadal function in CAH males.

**Subjects and methods:** The patient cohort comprised 30 males, aged 19–67 years, with 21-hydroxylase deficiency. Their fertility was compared with age-matched national population data. For the evaluation of social/sexual factors and hormone status, age-matched controls were recruited ( $n=32$ ). Subgroups of different ages (<30 years and older) and CYP21A2 genotypes (null (severe salt-wasting (SW)), I2splice (milder SW), and I172N (simple virilizing)) were also studied. Patients underwent testicular ultrasound examination ( $n=21$ ) and semen analysis ( $n=14$ ).

**Results:** Fertility was impaired in CAH males compared with national data ( $0.9 \pm 1.3$  vs  $1.8 \pm 0.5$  children/father,  $P<0.001$ ). There were no major differences in social and sexual factors between patients and controls apart from more fecundity problems, particularly in the I172N group. The patients had lower testosterone/estradiol ( $E_2$ ) ratio and inhibin B, and higher FSH. The semen samples were pathological in 43% (6/14) of patients and sperm concentration correlated with inhibin B and FSH. Testicular adrenal rest tumors (TARTs) were found in 86% (18/21). Functional testicular volume correlated positively with the testosterone/ $E_2$  ratio, sperm concentration, and inhibin B. Patients with pathological semen had increased fat mass and indications of increased cardiometabolic risk.

**Conclusions:** Fertility/fecundity was impaired in CAH males. The frequent occurrence of TARTs resulting in testicular insufficiency appears to be the major cause, but other factors such as elevated fat mass may contribute to a low semen quality.

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**Introduction**

Reported fertility in males with congenital adrenal hyperplasia (CAH) has ranged from normal (1) to severely impaired (2). The occurrence of testicular adrenal rest tumors (TARTs) is considered to be the main reason for fertility problems. Their typical location in the rete testis is associated with the risk of obstruction of the seminal ducts, with subsequent permanent testicular damage. Increased adrenal androgens due to not only undertreatment but also overtreatment with glucocorticoids, leading to gonadotropin suppression, may hamper fertility (3, 4).

The relative impact of different components involved is not evident from previous studies. One reason may be the different modes of evaluation used. Testicular function has been studied by measuring inhibin B and

serum gonadotropin levels, along with semen analysis and/or the number of biological children. TARTs have been diagnosed using palpation, ultrasound, or magnetic resonance imaging (1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12).

We have previously shown that reduced fertility in females with CAH is mainly attributable to social and sexual issues (13), but very little is known about these issues in males with CAH.

Thus, the aim of this study was not only to evaluate fertility, fecundity, and TARTs in adult CAH males, but also to investigate the social and sexual factors of importance for fertility. Moreover, younger and older patients, as well as different CYP21A2 genotypes, were compared to reveal the potential changes associated with age or disease severity.

## Subjects and methods

### Subjects

CAH males,  $\geq 19$  years of age, were mainly recruited from the two participating university hospitals. The diagnosis was confirmed by mutation analyses of the *CYP21A2* gene (31 patients) or the *HSD3B2* gene (one patient).

The data were divided into subgroups according to age as  $< 30$  years and older and according to the three most prevalent *CYP21A2* mutations as null, I2splice, and I172N. In compound heterozygotes, the mildest mutation defines the genotype group. Null is associated with the salt-wasting (SW) phenotype, I2splice is most often associated with the SW phenotype but some patients may escape SW, and I172N typically leads to simple virilizing (SV) CAH. Matched controls born on the same date as the patients were recruited from the National Population Registry. The only exclusion criterion was severe mental or psychiatric disturbance with inability to consent to the study. Age-matched national fertility data were also used (children/father) (14).

This study was approved by the Ethics Committees of the Karolinska Institute, Stockholm, and the University of Gothenburg, Göteborg, Sweden. All participants gave their written informed consent.

### Study protocol

Patients and controls were examined as outpatients at Karolinska University Hospital (22 patients and 22 controls), Stockholm, or Sahlgrenska University Hospital (ten patients and ten controls), Göteborg, Sweden. Serum and plasma samples were collected in the morning after an overnight fast before morning medication. All participants answered questionnaires concerning social and sexual issues, fertility, and fecundity. Fecundity problems were defined as attempting to become a father for  $> 1$  year. Patients underwent testicular ultrasound imaging and were also asked to deliver a semen sample. Published data from this cohort on body composition measured by dual-energy X-ray absorptiometry and cardiovascular risk factors (15) were used to evaluate whether differences could be found in these parameters between patients with normal and patients with pathological semen.

### Testicular examination

The testicles were only examined at one of the participating centers. This was done by manual palpation and ultrasound. An orchidometer was used to estimate the volume. The ultrasound examinations were performed by one physician (S Granberg) using a Voluson Expert 730 machine equipped with a

6–16 MHz real-time three-/four-dimensional linear probe (GE Healthcare Austria GmbH & Co. OG, Zipf, Austria). Total testicular, TART, and functional (total testicular–TART) volumes were estimated using the formula for a prolate ellipsoid (maximal length  $\times$  maximal width  $\times$  maximal depth  $\times 0.523$ ) (16).

### Semen analysis

Seminal fluid was collected after 1–4 days of ejaculatory abstinence. The analyses included assessment of semen volume, sperm concentration, total sperm count, motile and immotile spermatozoa, and morphology. Results were evaluated according to the World Health Organization (WHO) standard (17).

### Biochemical assays

Serum testosterone, sex hormone-binding globulin (SHBG), and dried blood spot 17-hydroxyprogesterone (17OHP, measured at 0800, 1400, 1900, 0100, and 0600 h), and 24-h urinary pregnanetriol were measured as described previously (15). Bioactive testosterone was calculated from total testosterone, SHBG, and albumin (18). Serum FSH, LH, estradiol ( $E_2$ ), and total prostate-specific antigen (PSA) and free PSA were measured by fluoroimmunoassay (Auto-Delfia, PerkinElmer, Waltham, MA, USA). FSH and LH had normal ranges of 1.0–10 U/l. Serum inhibin B was measured by ELISA (Beckman Coulter, Toronto, ON, Canada; normal range was 25–325 ng/l and detection limit was 10 ng/l). Plasma ACTH was measured on an advantage automatic immune analyzer and plasma renin by immunoradiometric assay (both from Nichols Institute Diagnostics, San Clemente, CA, USA).

### Statistical analysis

SigmaStat for Windows (Jandel Scientific, Erkarath, Germany) was used for data analysis. Results are presented as mean  $\pm$  s.d. if not otherwise stated. An unpaired *t*-test was used to compare two groups when values were normally distributed. Otherwise, the Mann–Whitney rank sum test was used and in these cases the median and range were reported. One-way ANOVA was used when comparing three groups with continuous variables and if normally distributed, followed by the *post-hoc* Bonferroni *t*-test; otherwise, the Kruskal–Wallis test was performed, followed by the *post-hoc* Mann–Whitney rank sum test and Dunn's method.  $\chi^2$  test or Fisher's exact test was used in frequency table calculations. All proportions were calculated discounting missing values. Spearman's correlation coefficient was used for correlation analyses. Statistical significance was set at  $P < 0.05$  and tendency at 0.05–0.10.

## Results

### Characteristics of the patients and controls

The characteristics of the patients have been described earlier (15). In brief, 32 males with CAH, aged 19–67 years, were examined. Thirty-one had 21-hydroxylase deficiency. Eighteen had been diagnosed with the SW phenotype (genotype groups: null,  $n=7$ ; I2splice,  $n=9$ ; I172N,  $n=1$ ; and C75R,  $n=1$ ). One patient had 3-beta-hydroxysteroid dehydrogenase (3 $\beta$ -HSD) type II deficiency (genotype: C75R). Twelve patients had been diagnosed with the SV form (I172N,  $n=8$ ; I2splice,  $n=3$ ; and P453S,  $n=1$ ). One SV patient had karyotype 46,XX (I2splice). Two patients had the non-classic (NC) phenotype with normal baseline ACTH levels (mildest mutation V281L and P105L+P453S). The patients with 3 $\beta$ -HSD deficiency and 46,XX were excluded from further analysis of the data but are separately described at the end of this section. Thus, 30 patients were included in the statistical analysis.

Glucocorticoids were taken by 93% (28/30) of the patients. One recently diagnosed 29-year-old man (I172N) took no medication and another with the NC phenotype (V281L) only used prednisolone when acutely ill. The most frequently used preparations were prednisolone (62%, 18/29) and hydrocortisone (17%, 5/29). The mean dose expressed in glucocorticoid equivalents was  $32.7 \pm 12.4$  mg using the conversion previously described (19). Urine pregnanetriol and blood 17OHP diurnal curves were similar for the different genotypes and age groups (15).

Most patients (87%, 26/30) received fludrocortisone, which was also received by most of the SV patients (82%, 9/11) and one of the NC patients. The SV and NC patients used fludrocortisone to minimize the glucocorticoid dose. None of the CAH males had experienced prostate problems. In the control group, a 67-year-old man had antiandrogen therapy after prostatectomy for cancer and was excluded in all calculations concerning testicular function, a 58-year-old man was operated on for benign prostatic hyperplasia, and a 45-year-old man had recurrent prostatitis.

### Fertility, fecundity, and sexuality

On comparing the number of biological children of CAH patients with age-matched national data, the fertility was low (in all patients,  $0.9 \pm 1.3$  vs  $1.8 \pm 0.5$ ; <30 years,  $0.1 \pm 0.3$  vs  $1.3 \pm 0.4$ ;  $\geq 30$  years,  $1.2 \pm 1.4$  vs  $2.0 \pm 0.3$ ; children/father,  $P < 0.001$  for all comparisons). In patients with classic 21-hydroxylase deficiency, the corresponding frequency was  $0.9 \pm 1.3$ . The reduction of fertility was similar in the three genotype groups (null, I2splice, and I172N) compared with age-matched national data ( $P < 0.001$ ). One NC man had three while the other had no children. However, compared with our investigated controls, fertility and

the age at the time of birth of the first child (not shown) were similar although more patients than controls had experienced fecundity problems (Table 1). Three patients had attempted to father but never succeeded, compared with one control, but one of them fathered a child 1 year after the investigation. The rate of marriage/cohabitation was similar in the entire patient group compared with controls. A propensity to increased frequency of marriage/cohabitation in older compared with younger subjects was only found in controls. A similar percentage of patients and controls had no partner (Table 1). One control had two adopted children.

The age at sexual debut and frequency of intercourse were similar between the groups (not shown), but the lifetime number of partners was lower in the total group of patients and in older patients compared with controls. One control declared that he was homosexual compared with none in the patient group. No bisexuality was reported. As shown in Table 2, there was no difference between the genotype groups regarding fertility, fecundity, and sexuality. However, compared with controls, the null group patients have had fewer partners, fewer I2splice patients were married/cohabitants, and fecundity problems were more common in the I172N group patients.

### Biochemical tests

Inhibin B was lower and FSH higher in all groups of CAH males compared with controls (Tables 1 and 2). The values were negatively associated in the entire patient cohort ( $r = -0.514$ ,  $P < 0.001$ ). Three patients had subnormal inhibin B and four had elevated FSH concentrations. No differences in LH levels were found. No subject displayed subnormal FSH or LH.

As expected, both older patients and controls had lower bioactive testosterone than the younger ones. An overall tendency to lower bioactive testosterone and higher E<sub>2</sub> in patients than in controls resulted in a significant decrease of the testosterone/E<sub>2</sub> ratio in all and older patients, with the latter also having a lower ratio than their younger counterparts (Table 1). SHBG was similar in all groups (not shown). Total PSA and free PSA concentrations were similar in all comparisons (not shown).

### Testicular examination

At manual palpation performed in 70% (21/30) of patients and controls, smaller testicular volumes were found in patients compared with controls (Table 1). Patients with the null mutation had smaller testicles than those with other genotypes or controls. A unilateral tumor was palpated in one patient and multiple bilateral tumors in another (Table 1). However, with the aid of ultrasound (completed in 70% (21/30)), 86% (18/21) were shown to have TARTs, which were

**Table 1** Results of fecundity, fertility, and sexuality evaluations, biochemical tests, and testicle palpation in adult male patients with CAH, also divided into <30 years of age or more and age-matched male controls (mean $\pm$ S.D. or median and range).

	Patients (n=30)	Controls (n=32)	P value	Patients <30 years (n=9)	Controls <30 years (n=10)	P value	Patients ≥30 years (n=21)	Controls ≥30 years (n=22)	P value
Age (years)	35.7 $\pm$ 11.4	36.5 $\pm$ 11.9	NS	23.6 $\pm$ 3.2	24.4 $\pm$ 3.3	NS	40.9 $\pm$ 9.5 <sup>#</sup>	42.0 $\pm$ 6.6 <sup>#</sup>	NS
Fertility and sexuality									
Children (n)	0 (0–5) (0.9 $\pm$ 1.3)	0 (0–4) (1.0 $\pm$ 1.2)	NS	0 (0–1) (0.1 $\pm$ 0.3)	0 (0–1) (0.1 $\pm$ 0.3)	NS	1.0 (0–5)* (1.2 $\pm$ 1.4)*	1.5 (1–4) <sup>†</sup> (1.4 $\pm$ 1.3) <sup>†</sup>	NS
Fecundity problems <sup>a</sup>	6/28	1/32	0.041	2/9	0/10	4/19	4/22	4/22	NS
Married/cohabitant	14/30	22/32	NS	3/9	4/10	NS	11/21	18/22 <sup>s</sup>	0.003
No partner	11/01	8/32	NS	5/9	5/10	NS	7/21	3/22 <sup>s</sup>	NS
Partners (n)	3.5 (0–100)	9.0 (1–50)	0.038	3.8 (0–38)	3.0 (1–21)	NS	3.5 (0–100)	13 (2–50)*	0.028
Biochemical tests									
FSH (U/l)	5.2 (1.5–23)	3.3 (1.6–6.7)	0.014	4.3 $\pm$ 2.5	2.9 $\pm$ 1.2	NS	5.2 (1.5–23)	3.3 (1.6–6.7)	0.022
Inhibin B (ng/l)	135 $\pm$ 74	211 $\pm$ 84	<0.001	155 $\pm$ 63	209 $\pm$ 84	NS	126 $\pm$ 78	210 $\pm$ 87	0.002
LH (U/l)	3.5 (1.1–22)	4.3 (1.5–6.2)	NS	3.6 (1.1–15)	4.4 (1.5–6.1)	NS	3.4 (1.2–22)	4.3 (1.9–6.2)	NS
Bioactive testosterone (nmol/l)	2.3 (0.5–7.7)	2.6 (1.2–6.7)	0.074	3.6 $\pm$ 1.9	4.1 $\pm$ 1.5	NS	1.9 $\pm$ 0.9 <sup>f</sup>	2.5 $\pm$ 1.2 <sup>t</sup>	0.082
E <sub>2</sub> (pmol/l)	97 $\pm$ 50	79 $\pm$ 25	0.066	91 $\pm$ 45	92 $\pm$ 36	NS	102 $\pm$ 53	74 $\pm$ 18	0.024
Testos/E <sub>2</sub> (nmol/pmol)	0.18 $\pm$ 0.09	0.22 $\pm$ 0.07	0.019	0.23 $\pm$ 0.11	0.23 $\pm$ 0.09	NS	0.15 $\pm$ 0.06*	0.22 $\pm$ 0.06	
Testicular palpation									
Size (cm <sup>3</sup> )	18.1 $\pm$ 4.2	20.7 $\pm$ 3.8	0.049	18.1 $\pm$ 2.9	19.1 $\pm$ 3.3	NS	21.6 $\pm$ 3.9	21.6 $\pm$ 3.9	0.057
Tumors	2/21	0/23	NS	1/8	0/8	NS	1/13	0/15	NS

\*P<0.05, <sup>†</sup>P<0.01, <sup>#</sup>P<0.001, and <sup>s</sup>P=0.05–0.099 compared with younger counterparts (patients or controls respectively). NS, non-significant; testos, testosterone; E<sub>2</sub>, estradiol; F, free; partners, partners during lifetime.

<sup>a</sup>Fecundity problems defined as trying to father a child for >1 year.

bilateral in 72% (13/18) of cases. An image illustrating a TART from one of the patients is shown in Fig. 1. No significant difference was found in TART frequency and number, total TART volume, or functional testicular volume between genotypes (Table 3). Two patients with genotypes associated with very mild disease (V281L and P105L+P453S) had small TARTs (0.31–0.38 cm<sup>3</sup>).

Patients with the largest TARTs (n=8, volume >1 cm<sup>3</sup>) had similar age and fat mass but higher lean mass (19.5 $\pm$ 2.4 vs 16.6 kg/m<sup>2</sup>, P=0.025) and shorter height (162 $\pm$ 8 vs 173 $\pm$ 6 cm, P=0.011) than those with no or very small TARTs (n=7, volume <0.2 cm<sup>3</sup>). Poor treatment control, arbitrarily defined as urinary pregnanetriol  $\geq$ 100  $\mu$ mol/24 h and/or a 24-h median 17OHP value  $\geq$ 100 nmol/l, was found in three of the eight patients with larger TARTs but in none of those with no or very small TARTs.

Two patients had undergone previous testicular surgery for suspected malignancy. In both cases, the histopathology was interpreted as benign Leydig cell tumors. One 41-year-old SW patient (I2splice) had a testicular tumor extirpated at 12 years of age. He now had one small tumor in each testicle (volume 0.11 cm<sup>3</sup>), normal semen, and two biological children. In a 34-year-old SW patient (I2splice) with two biological children, a tumor was removed before conception. He now showed multiple bilateral tumors. One 23-year-old SV patient (I2splice) with TARTs had been operated on for testicular retention at age 17. No testicular intervention was reported in any of the controls.

### Semen analysis

Semen for analysis was obtained from 14 of the 30 CAH males. Fecundity problems tended to be more common in those who delivered a semen sample than in those who declined (5/14 vs 1/16, P=0.072). The mean ages were similar (37.7 $\pm$ 12.7 vs 35.6 $\pm$ 11.9 years).

Considering all WHO criteria, six of the 14 semen samples were pathological. The results were similar in younger and older patients (not shown). A lower sperm count, concentration, and motility in I172N compared with the I2splice group did not reach statistical significance in this limited material (Table 3). Only one patient in the null group had an analyzed sample and it was pathological. One 39-year-old patient with SV (I172N) had severe teratozoospermia with impaired motility and a previously performed intracytoplasmic sperm injection (ICSI) resulting in one child.

We found that inhibin B levels, regarded as a marker of spermatogenesis, were negatively correlated to body mass index (BMI; r=−0.509, P=0.004) in the controls and demonstrated a similar trend in the patients (r=−0.317, P=0.087). Hence, body

**Table 2** Results of fecundity, fertility, and sexuality evaluations, biochemical tests, and testicle palpation in adult male patients with the three most common CYP21A2 genotypes and male controls (mean  $\pm$  S.D. or median and range).

	Null (n=7)	P value vs controls	I2splice (n=11)	P value vs controls	I172N (n=9)	P value vs controls	Controls (n=32)	P value genotypes
Age (years)	34.5 $\pm$ 9.5	NS	31.6 $\pm$ 9.4	NS	41.0 $\pm$ 14.9	NS	36.5 $\pm$ 11.9	NS
Fertility and sexuality								
Children (n)	0 (0–3) (1.0 $\pm$ 1.3)	NS	0 (0–3) (0.58 $\pm$ 1.1)	NS	1 (0–5) (1.1 $\pm$ 1.6)	NS	0 (0–4) (1.0 $\pm$ 1.2)	NS
Fecundity problems <sup>a</sup>	0/5	NS	2/11	NS	3/7	0.014	1/32	NS
Married/cohabitant	4/7	NS	3/11	0.031	5/8	NS	22/32	NS
No partner	3/7	NS	6/11	NS	2/8	NS	8/32	NS
Partners (n)	1–3 $\pm$ 1.5	0.031	3.0 (0–100)	NS	4.8 (0–60)	NS	9.0 (1–50)	0.081
Biochemical tests								
FSH (U/l)	5.2 (1.5–21)	0.006	5.2 (1.8–23)	0.029	3.8 (1.6–12)	0.049	3.3 (1.6–6.7)	NS
Inhibin B (ng/l)	126 $\pm$ 63	0.019	131 $\pm$ 102	0.016	120 $\pm$ 42	0.004	211 $\pm$ 84	NS
LH (U/l)	2.5 (1.2–22)	NS	5.0 $\pm$ 2.7	NS	3.4 (1.1–11)	NS	4.3 (1.5–6.2)	NS
Biactive testosterone (nmol/l)	2.44 (0.5–7.7)	NS	1.8 (0.9–5.2)	NS	2.2 (0.9–3.8)	0.092	2.6 (1.2–6.7)	NS
E <sub>2</sub> (pmol/l)	77 (56–298)	0.063	87 $\pm$ 39	NS	90 $\pm$ 26	NS	79 $\pm$ 25	NS
Testos/E <sub>2</sub> (nmol/pmol)	0.16 $\pm$ 0.09	0.021	0.21 $\pm$ 0.10	NS	0.17 $\pm$ 0.06	0.072	0.22 $\pm$ 0.07	NS
Testicular palpation								
Size (cm <sup>3</sup> )	13.5 $\pm$ 3.0	0.002	19.7 $\pm$ 3.2	NS	18.0 $\pm$ 4.8	NS	20.7 $\pm$ 3.8	0.042 <sup>b</sup>
Tumors	2/4	0.017	0/9	NS	0/5	NS	0/23	0.019 <sup>c</sup>

NS, non-significant; testos, testosterone; E<sub>2</sub>, estradiol; F, free.<sup>a</sup>Fecundity problems defined as trying to father a child for >1 year.<sup>b</sup>Post-hoc null vs I2splice, P=0.040.<sup>c</sup>Post-hoc null vs I2splice, P=0.077.

composition data and other markers for cardiovascular risk were compared in males with pathological and normal semen. Males with pathological semen had a higher truncal and total fat mass, total fat/lean mass ratio, and heart rate, in spite of similar 17OHP levels and corticosteroid doses (Table 4).

### Associations among TARTs, sperm number and quality, and the gonadotropin-gonadal steroid axis

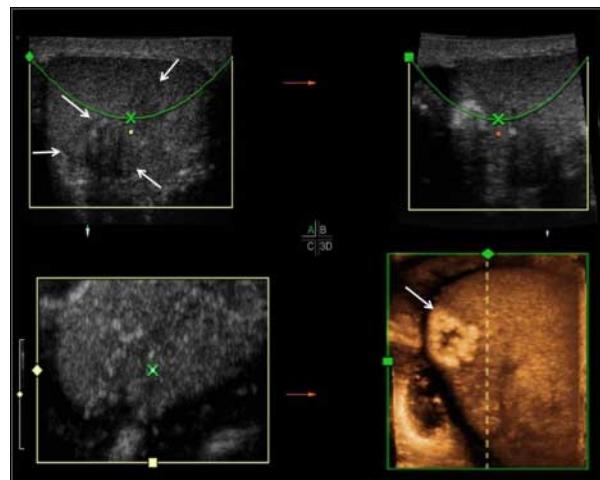
As shown in Fig. 2, total functional testicular volume was positively correlated with inhibin B levels and sperm concentration. There was also a trend to a positive association with sperm volume ( $r=0.60$ ,  $P=0.059$ ) and a significant positive correlation with the testosterone/E<sub>2</sub> ratio ( $r=0.50$ ,  $P=0.029$ ). We also found the expected positive associations between inhibin B total functional testicular volume and the sperm count ( $r=0.64$ ,  $P=0.014$ ) and concentration (Fig. 2) and with normal morphology ( $r=0.48$ ,  $P=0.061$ ). FSH showed a negative correlation with sperm count ( $r=-0.53$ ,  $P=0.047$ ) and concentration ( $r=-0.70$ ,  $P=0.004$ ). The total TART volume tended to be negatively associated with height ( $r=-0.44$ ,  $P=0.056$ ) and was positively correlated with LH ( $r=0.41$ ,  $P=0.046$ ). There was no correlation with other hormones of the gonadotropin-testicular axis. No correlation was demonstrated between total TART volume or functional testicular volume and age, glucocorticoid dose, plasma renin, and ACTH concentrations (not shown).

### Male with 3 $\beta$ -HSD type II deficiency

The patient with 3 $\beta$ -HSD deficiency was 21 years old, had male external genitalia with a micropenis, lived alone, and had no children. On testicular palpation, bilateral multiple tumors were found and ultrasound showed a very small total functional volume (1.9 cm<sup>3</sup>) due to large bilateral TARTs measuring 25.6 cm<sup>3</sup>. A tumor measuring 2.4 $\times$ 1.8 $\times$ 1.5 cm, with a weight of 3.2 g, was extirpated. He had no sperm in the semen sample, but a testicular biopsy was able to retrieve  $\sim$ 100 severely abnormal sperms, which were saved for future ICSI. He had poor control with high DHEAS, and his FSH and LH were also high while inhibin B was low.

### Male with karyotype 46,XX and 21-hydroxylase deficiency

The 46,XX CAH male was 61 years old and had a 4 cm phallus. He was diagnosed at age 7 when ovaries and a uterus had been found and was subsequently extirpated, following which he underwent glucocorticoid and testosterone replacement. He was married and had adopted two children.



**Figure 1** Illustration of the steps in the 3D inversion rendering process of right testicle of a 30-year-old male with CAH (genotype null/null) with two children. Acquisition in the three orthogonal planes and the rendered volume in the bottom right box. By eliminating the surrounding non-pertinent structures with the electronic scalpel, the structures of interest were left as the final inverted and rendered volume. In this case, it could be seen that the tumor was calcified and had a cystic structure (right bottom corner, the tumor marked with an arrow). In the left upper box, the tumor is marked with arrows (the size of the tumor was measured to be:  $2.6 \times 1.1 \times 0.9$  cm, corresponding to a volume of  $1.4 \text{ cm}^3$ ). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-11-0828>.

## Discussion

This study confirms that fertility is reduced in males with classic CAH. They also had fecundity problems more often than control men. TARTs were present in the majority of patients; hormonal evaluation demonstrated disturbed testicular function involving both Sertoli and Leydig cells, and in about half of the patients who had semen analysis performed, abnormal semen was found. Psychosocial and sexual issues were also

investigated, but their impact on overall fertility rate appears modest.

The fertility rate was reduced by one half compared with national data and was impaired in all genotype groups associated with classic CAH. No obvious reason was found in the patients' social or sexual life. Although the patients were less experienced than controls in terms of number of sexual partners through life, the age at sexual debut, the rate of marriage/cohabitation, and the present coitus frequency were similar in patients and controls. More fecundity problems were reported, however, by the men in the I172N group. This may be due to metabolic factors (see below).

One reason for the decreased birth rate in females with CAH is increased homo- and bisexuality, with fewer individuals living in heterosexual relationships (13, 20). Among the male patients, none reported homo- or bisexuality. This is in keeping with the only previous report on sexual orientation studying nine CAH males (21).

Most likely, the predominant reason for reduced fertility is the frequent occurrence of TARTs, which were found in 86% of the examined patients, similar to the highest frequency reported previously (6). Others report prevalences of 0–67%, which probably reflects differences in the mode of detection and age of the patients (1, 2, 4, 5, 7, 9, 10, 11, 12). TARTs have been demonstrated in children at a frequency of slightly above 20% and found even in children 6 years of age (22, 23). Autopsy data have indicated TARTs in CAH boys of a few weeks of age (24).

We found TARTs in all genotype groups. Even both of our NC patients who had normal ACTH levels had small TARTs, which has previously been reported only with clearly elevated ACTH or angiotensin II (AII) (3). In contrast, a recent study found no case of TART in the I172N or the NC genotypes (9). Others have, however, also reported TARTs in I172N patients (4, 5, 22), and in patients with SV (11). Another recent report found

**Table 3** Testicular ultrasound exams and semen samples from adult patients with CAH and the three most common CYP21A2 genotypes (mean  $\pm$  s.d. or median and range).

	Patients	Null	I2splice	I172N	P value genotypes
Ultrasound					
Total volume ( $\text{cm}^3$ )	$n=21$ $22.7 \pm 7.5$	$n=4$ $18.7 \pm 9.0$	$n=10$ $24.6 \pm 6.0$	$n=4$ $24.4 \pm 11.6$	NS
TART present	$18/21$	$4/4$	$8/10$	$4/4$	NS
TART ( $n$ )	$2.0 (0-8)$	$2.0 (1-5)$	$2 (0-8)$	$1.5 (1-2)$	NS
Total TART ( $\text{cm}^3$ )	$0.5 (0-8.7)$	$2.9 (1.9-4.6)$	$0.2 (0-8.7)$	$0.5 (0.2-4.3)$	0.083
Total functional ( $\text{cm}^3$ )	$20.4 (7.1-36.7)$	$15.6 \pm 8.1$	$24.4 \pm 5.7$	$23.0 \pm 9.8$	NS
Semen sample	$n=14$	$n=1$	$n=6$	$n=6$	
Volume (ml)	$2.3 \pm 0.6$	1.5	$2.3 \pm 0.5$	$2.2 \pm 0.6$	NS <sup>a</sup>
Sperm count ( $\times 10^6$ )	$151 \pm 112$	105	$220 \pm 146$	$88 \pm 19$	0.053 <sup>a</sup>
Sperm conc. ( $\times 10^6/\text{ml}$ )	$70 \pm 54$	70	$99 \pm 71$	$44 \pm 18$	0.094 <sup>a</sup>
Motile sperm (%)	$53 \pm 21$	30	$64 \pm 11$	$42 \pm 23$	0.060 <sup>a</sup>
Normal morphology (%)	$3.2 \pm 2.8$	1.0	$4.5 \pm 3.4$	$2.2 \pm 1.9$	NS <sup>a</sup>
Pathological semen	$6/14$	1/1	$3/7$	$2/5$	NS

NS, non-significant; TART, testicular adrenal rest tumor.

<sup>a</sup>Only one male null/null patient provided a semen sample, thus comparisons were only performed between I2splice and I172N.

**Table 4** Patient characteristics and differences found in body composition, heart rate, blood pressure, and biochemical tests in adult CAH male patients with pathological and normal semen (mean  $\pm$  s.d. or median and range).

	Pathological (n=6)	Normal (n=8)	P value
Age (years)	40.3 $\pm$ 15.1	35.8 $\pm$ 11.2	NS
Phenotype	4SW/2SV	4SW/2SV	NS
HCeq/m <sup>2</sup> (mg/m <sup>2</sup> )	18.9 $\pm$ 8.4	15.4 $\pm$ 2.2	NS
BMI (kg/m <sup>2</sup> )	29.4 $\pm$ 6.3	25.3 $\pm$ 4.1	NS
Total fat mass (%)	32.0 $\pm$ 8.2	24.3 $\pm$ 5.7	0.049
Truncal fat mass (%)	35.2 $\pm$ 9.7	25.9 $\pm$ 7.0	0.048
Total fat/lean mass	0.5 $\pm$ 0.2	0.3 $\pm$ 0.1	0.032
24-h BP (mmHg)	134 $\pm$ 6/79 $\pm$ 5	123 $\pm$ 11/74 $\pm$ 5	0.062/0.099
24-h heart rate	81 $\pm$ 5	73 $\pm$ 5	0.012
Fecundity problems <sup>a</sup>	5/6	0/8	0.003
FSH >10 (U/l)	2/6	0/8	NS
Inhibin B (ng/l)	157 $\pm$ 57	167 $\pm$ 102	NS
Testosterone (nmol)	12.5 $\pm$ 3.2	17.8 $\pm$ 6.2	0.064
Testos/E <sub>2</sub> (nmol/pmol)	0.17 $\pm$ 0.04	0.19 $\pm$ 0.07	NS
S-TG (mmol/l)	1.5 $\pm$ 1.0	0.9 $\pm$ 0.3	0.095

NS, non-significant; HCeq, hydrocortisone equivalents; TG, triglyceride; BP, blood pressure; testos/E<sub>2</sub>, testosterone/estradiol ratio.

<sup>a</sup>Fertility problems defined as attempting to father a child for >1 year.

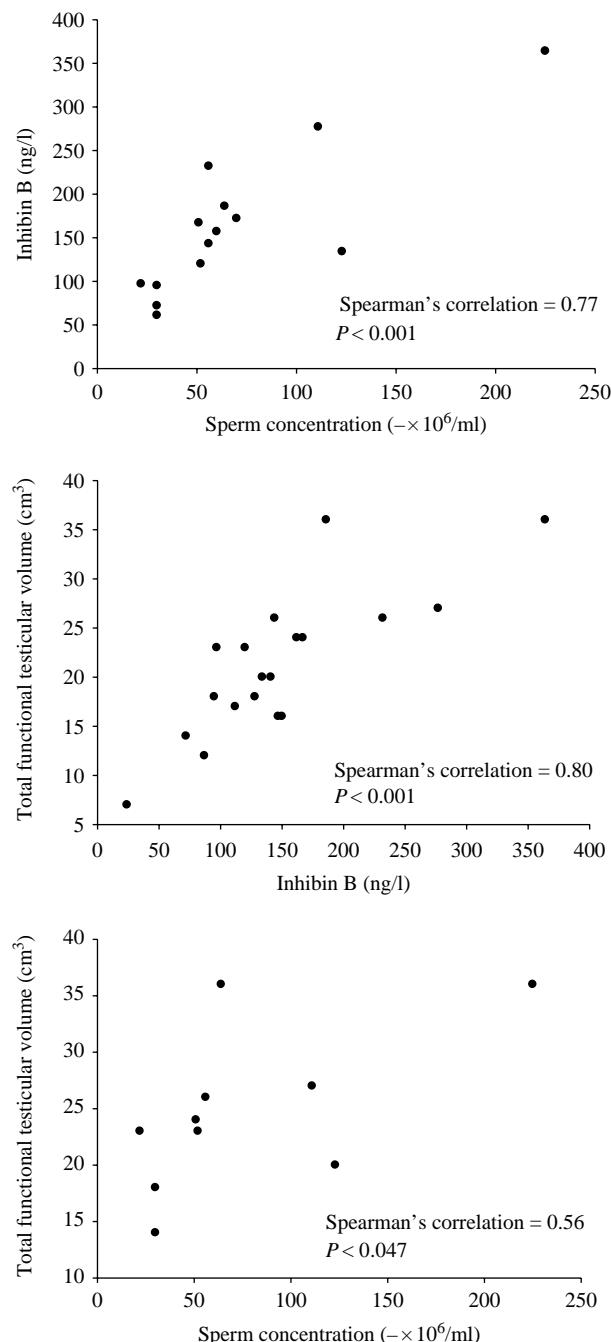
TARTs exclusively in SW patients (12). The higher mean age of our patients compared with many of the previous studies may explain some of the differences. Only 10% of TARTs were found by clinical examination in our cohort and it has been stated that usually only TARTs > 2 cm are detectable by palpation due to being buried within the testis (3). Consequently, all CAH males, including those with the milder pheno- and genotypes, should be examined periodically by testicular ultrasound and not only the classic ones.

It is alarming that two (6.7%) of the CAH males had undergone testicular surgery for suspected malignancy with histology showing TARTs. Almost the same frequency of unnecessary testicular surgery for presumed malignancy has recently been reported from a large cohort of CAH males (6.2%, 4/65) (10). Thus, an increased awareness and understanding of TARTs is needed.

There were associations in favor of a negative impact of TARTs on fertility. Total functional testicular volume was positively correlated with sperm parameters and inhibin B. Semen quality has recently been reported to be very poor in CAH males, with 100% being pathological if all the WHO criteria were considered (4). Our patients appeared less affected; using the same criteria, 43% of our CAH males had abnormal semen.

The results of several studies point to an increased likelihood of abnormal semen parameters among overweight men and obesity is associated with a higher incidence of male factor infertility (25). In large cohorts, decreased sperm quality was found already in overweight (BMI 25–30 kg/m<sup>2</sup>) compared with normal weight men (26). One of the proposed mechanisms is that increased estrogens due to aromatization of androgens in the adipose tissue can hamper fertility (25). Elevated fat mass is often found in CAH (27, 28),

and our CAH males with abnormal semen demonstrated increased total and abdominal body fat, and fat to lean mass ratio compared with CAH males with normal semen. Overall, our patients had a lower testosterone/E<sub>2</sub> ratio although the difference in these parameters between individuals with normal and pathological semen did not reach significance in the



**Figure 2** Correlation between inhibin B (upper panel), total functional testicular volume (lower panel) and sperm concentration, and total functional testicular volume and inhibin (middle panel) in adult males with CAH due to 21-hydroxylase deficiency.

present small sample. The I172N genotype group tended to have a lower sperm quality compared with the I2splice patients, perhaps reflecting their previously reported negative cardiometabolic profile (15). Metabolic risk has been associated with decreased fertility and impaired sperm quality (29). None of the patients showed suppression of gonadotropins, which could be expected with extreme over- or undertreatment with glucocorticoids (3, 4).

Apart from conventional infertility treatment, there are few specific measures to offer CAH men with fertility problems. Weight loss may improve fertility in obese men (25) and normal weight should be aimed at. TARTs possess receptors for both ACTH and AII, and it has been hypothesized that high levels due to undertreatment with corticosteroids may enhance their growth (30). We found no correlation between TART volume and current ACTH or renin levels. However, subjects with large TARTs were shorter and had higher lean mass compared with those with no or very small TARTs. Almost 40% of patients with large TARTs had very high levels of urinary pregnanetriol and blood 17OHP, thereby indicating both poor control during adolescence and later. Increased glucocorticoid doses can reduce the size of the TARTs in the early stages, but continued growth is also seen when ACTH levels are suppressed. It remains unknown whether this is due to persistent stimulation via AII receptors or by other mechanisms as the effect of increased mineralocorticoid doses has not been systematically studied. Unfortunately, higher doses of glucocorticoids and mineralocorticoids will most certainly increase the frequency and severity of side effects (10, 15, 28, 31, 32). In later stages, testicular surgery may sometimes be helpful, but in the end, when damage is irreversible, treatment options are very limited (3). Preservation of sperm could be considered before the TARTs have grown too much.

Fertility in 3 $\beta$ -HSD deficiency has not been studied (33), but our patient was infertile with no sperm in his semen sample. Biopsy revealed a few severely affected sperms; ultrasound and surgery revealed virtually no functional testicular volume.

The major limitation of this study is its size despite being larger and including older CAH males than many other studies. Negative findings must therefore be interpreted with caution. Moreover, our controls had lower fertility than expected. Many of our patients did not collect a semen sample and a comparison with those who did indicated a selection bias as fecundity issues tended to be more prevalent in the latter group. Hence, those with problems appeared more motivated to produce a sample.

In conclusion, impaired fertility and fecundity were detected in adult men with CAH. Sexual and social issues appeared to have limited impact on fertility. The most obvious cause is the presence of TARTs, but other causes may contribute. Decreased semen quality was prevalent and those with pathological semen also had

higher fat mass. The male with 3 $\beta$ -HSD deficiency was infertile. In spite of these findings, most males with CAH due to 21-hydroxylase deficiency who wish to be fathers seem to succeed eventually.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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