

Gonadal Function in Adult Male Patients with Congenital Adrenal Hyperplasia

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1 **Gonadal Function in Adult Male Patients with Congenital Adrenal Hyperplasia**

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51 **Keywords:** congenital adrenal hyperplasia; gonadal function; fertility; testicular adrenal rest tumor;

52 hypogonadism

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54

55 **Abstract**

56 **Context:** Current knowledge on gonadal function in Congenital Adrenal Hyperplasia (CAH) is mostly
57 limited to single center/country studies enrolling small patient numbers. Overall data indicate that
58 gonadal function can be compromised in men with CAH. ~~Gonadal function can be compromised in~~
59 ~~male patients with congenital adrenal hyperplasia (CAH), however previous studies have been~~
60 ~~limited to reports from a single center/country or small patient numbers.~~

61 **Objective:** To determine gonadal function in men with CAH within the European “dsd-LIFE” cohort.

62 **Design:** Cross-sectional clinical outcome study, including retrospective data from medical records.

63 **Methods:** Fourteen academic hospitals included 121 men with CAH aged 16-68 years. Main outcome
64 measures were serum hormone concentrations, semen parameters, and imaging data of the testes.

65 **Results:** At the time of assessment, ~~19/83~~ 14/69 patients had a serum testosterone concentration
66 level below the reference range; 8 7 of those were hypogonadotropic, 10 6 normogonadotropic, and
67 1 hypergonadotropic. In contrast, in the presence of among the patients with normal serum
68 testosterone (~~64/83~~ 55/69), 5 4 patients were hypogonadotropic, 50 44 normogonadotropic, and 9 7
69 hypergonadotropic. The association of decreased testosterone with reduced gonadotropin
70 concentrations (Odds Ratio (OR)=~~8.0 [2.2-29.6]~~ 12.8 [2.9-57.3]) was weaker than the association
71 between serum androstenedione/testosterone ratio ≥ 1 and reduced gonadotropin concentrations
72 (OR=~~16.8 [2.0-142.5]~~ 39.3 [2.1-732.4]). Evaluation of sperm quality revealed decreased ~~Decreased~~
73 sperm concentrations (15/39), ~~decreased~~ motility (13/37), and abnormal morphology (4/28) were
74 also observed. Testicular adrenal rest tumor (TART)s were present in 39/80 patients, with a higher
75 prevalence in patients with the most severe genotype (14/18), and in patients with increased current
76 17-hydroxyprogesterone (~~12/18~~ 20/35) or androstenedione (~~16/26~~ 12/18) serum concentrations.
77 Forty-three children were fathered by 26/113 patients.

78 **Conclusions:** Men with CAH have a high risk of developing hypothalamic-pituitary-gonadal
79 disturbances and spermatogenic abnormalities. Regular assessment of endocrine gonadal function
80 and of **imaging for** TART development ~~by imaging~~ are recommended, in addition to measures for
81 fertility protection.

82 Introduction

83 Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder resulting in impaired
84 adrenocortical steroid synthesis by several enzyme deficiencies. The most common form (>95%) is
85 21-hydroxylase deficiency (21OHD) with an incidence of 1:15 000, leading to glucocorticoid and often
86 also mineralocorticoid deficiency **in combination with androgen excess** ^{1,2}.

87 Reported fertility and fecundity in men with CAH **on routine steroid replacement therapy** range from
88 normal to severely impaired. Fertility can be compromised due to primary (hypergonadotropic)
89 hypogonadism or central (hypogonadotropic) hypogonadism ³⁻¹¹. In addition, **reduced** fertility and
90 fecundity **rates** ~~problems~~ in CAH can be caused by psychosexual factors ⁴. ~~One of the commonest~~
91 ~~complications in men with CAH is the presence of Testicular Adrenal Rest Tumor (TART)s, which can~~
92 ~~cause disturbances of gonadal function, including mechanical obstruction of the seminiferous~~
93 ~~tubules. The reported prevalence of TART ranges between 12.5% and 94% of the populations~~
94 ~~studied.~~ Central or secondary hypogonadism is defined as decreased testosterone concentrations in
95 combination with either low or low-normal LH or FSH concentrations. In ~~patients~~ **men** with CAH,
96 secondary hypogonadism is most likely ~~to be~~ caused by the suppressive effect of elevated adrenal
97 androgens (that are aromatized to estrogens) on the hypothalamic-pituitary-gonadal (HPG)-axis ⁶.
98 Differentiation between gonadal and adrenal testosterone is difficult, complicating the diagnosis of
99 hypogonadism in ~~patients~~ **men** with CAH. **One of the commonest complications in men with CAH is**
100 **the presence of Testicular Adrenal Rest Tumor (TART)s, which can cause disturbances of gonadal**
101 **function, including mechanical obstruction of the seminiferous tubules. The reported prevalence of**
102 **TARTs ranges between 12.5% and 94% in the populations studied** ^{4-10, 12-22}.

103 Until now, the data on fertility outcome in men with CAH are scarce. ~~Available data are~~ ³⁻¹¹ **and** often
104 derived from studies with patients from a single center or country. Our aim was to study gonadal
105 function in a large European multi-center cohort of male patients with CAH by evaluating hormone
106 concentrations, semen parameters, and TART frequency.

107 **Subjects and Methods**

108 **Subjects**

109 dsd-LIFE is a cross-sectional clinical outcome study of individuals with disorders/differences of sex
110 development (DSD). Fourteen study centers in 6 European countries (France (n=4), Germany (n=4),
111 United Kingdom (n=1), Poland (n=2), Sweden (n=1), and the Netherlands (n=2)) included former and
112 current patients as participants from February 2014 - September 2015. In addition to DSD
113 participants, 121 male participants with CAH (46XY karyotype) aged 16-68 years were recruited as
114 they may face similar clinical challenges as DSD patients, including sex hormone imbalances and
115 fertility problems, although male patients with CAH do not fit into the classification of DSD. Written
116 informed consent was obtained from all participants and/or their parents, with assent of minors.
117 Ethical approvals were obtained as appropriate for each country, e.g. Ethics Commission of the
118 Charité Universitätsmedizin; reference number EA2/069/13. For. The theoretical and methodological
119 framework of the dsd-LIFE study **have been published in detail elsewhere** see Röhle 2017 et al.²³.
120 Patients were investigated in their local treatment center. Cross-sectional data were obtained for
121 serum hormone concentrations, semen parameters and testicular imaging. The genotype of patients
122 with 21OHD was classified into genotype groups null, A, B, and C²⁴. ~~Patients were also classified into~~
123 ~~salt-wasting (SW), simple virilizing (SV) or non-classical (NC) based on their main symptoms and time~~
124 ~~of diagnosis~~ General patient characteristics and clinical parameters included: country of inclusion,
125 age, age at diagnosis, CAH genotype and phenotype, socioeconomic status, and obesity, as well as
126 height, weight, and BMI throughout the years (at diagnosis, 9 months old, 6 years old, Tanner stage
127 2, 16 years old, and current age). **Patients' educational levels was established according to the EU**
128 **classification. We combined the standardized ES-ISCED (international standard classification of**
129 **education) scale to Low (ES-ISCED I = less than lower secondary and ES-ISCED II = lower secondary);**
130 **medium (ES-ISCED IIIb = lower tier upper secondary; ES-ISCED IIIA = upper tier upper secondary; ES-**
131 **ISCED IV = advanced vocational, sub-degree) and high (ES-ISCED V1 = lower tertiary education, BA**

132 level; ES-ISCED V2 = higher tertiary education, \geq MA level). Data was collected during medical
133 examination at study inclusion (cross-sectional) and retrieved from medical records (retrospective
134 data).

135 **Hormonal analysis**

136 Blood samples were taken during day time, but mostly in the morning, before intake of the
137 glucocorticoid medication ²³. Total testosterone, SHBG, LH, FSH, inhibin B, AMH, androstenedione,
138 17-hydroxyprogesterone (17OHP) concentrations, and renin/plasma renin activity were measured in
139 the local hospital laboratory and compared to local references. Values are reported in SI or
140 international units and reported as "below reference range", "within reference range", "above
141 reference range up to twice the upper limit", and "more than twice the upper limit of the reference
142 range". To increase the number of patients per category, we combined the latter 2 categories into
143 the category "above reference range".

144 The serum androstenedione/testosterone ratio (AD/T) was calculated and divided into normal (<0.5 ;
145 interpreted as testosterone mainly of testicular origin), ≥ 0.5 and <1 (significant fraction of
146 testosterone is of adrenal origin), and ≥ 1 (testosterone mainly of adrenal origin) as suggested by
147 others ²⁵.

148 ~~Three patients were excluded from part of the analyses as they received testosterone substitution,~~
149 ~~which directly affects testosterone and gonadotropin concentrations. Two of these patients had data~~
150 ~~on TART available; these are described in the results section, but were otherwise excluded from~~
151 ~~further analyses.~~

152 **Semen analysis**

153 Semen analysis was performed by the local hospital laboratory and interpreted in accordance with
154 the 2010 World Health Organization criteria ²⁶, including sperm concentration (lower reference limit

155 (LRL: 15×10^6 /mL), motility (LRL: 40%), morphology (LRL: 4%), vitality (LRL: 58%), and volume (LRL:
156 1.5 mL).

157 **Imaging of testes**

158 At the study visit, 68 patients (56.2%) underwent testicular ultrasound. The presence of TART at the
159 age of 16 years was also reported retrospectively (in 30/68 patients with cross-sectional TART data).
160 In addition, retrospective data were available for 12 participants based on ultrasound findings or MRI
161 (n=11) and on histological findings (n=1).

162 **Paternity**

163 Data about paternity and relationships were collected from the dsd-LIFE questionnaires ²³.

164 **Medication and estimation of metabolic control in the past**

165 Patients used different formulations of glucocorticoids, including hydrocortisone, prednisone,
166 prednisolone, and dexamethasone. Furthermore, we converted all All glucocorticoid preparations
167 were converted to hydrocortisone equivalents for comparison, using the following factors for the
168 glucocorticoid equivalent dose: 1 (hydrocortisone), 4 (prednisone or prednisolone), 30
169 (dexamethasone), and 15 (fludrocortisone)²⁷. We also calculated mineralocorticoid equivalent dose
170 using the following factors: 1 (hydrocortisone), 0.8 (prednisone or prednisolone), 0 (dexamethasone),
171 and 200 (fludrocortisone)²⁷. In addition to the serum 17OHP concentrations presented in the section
172 hormonal analysis, we also assessed metabolic control by a subjective rating, of metabolic control of
173 the local examining physician at 5 different time points: at diagnosis, at the age of 9 months, at
174 Tanner stage 2, at age 16 years and at study inclusion, using the following scores: "poor",
175 "moderate", "good", "excellent" or "unknown".

176 **Statistical Analysis**

177 SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) was used for all analyses. Descriptive analyses were
178 performed for all variables. Depending on normality, mean and 95% confidence intervals (95%CI) or
179 median and interquartile ranges (IQR) were calculated. We compared patients with values below or
180 above reference range to patients with normal values (within the reference range). Odds ratios (OR)
181 with 95%CI were calculated if at least 3 cases were present in both subgroups. If any cell count in the
182 contingency table was zero, OR and 95%CI were calculated manually by using a continuity correction
183 (+0.5 in each cell).

184 Missing data were evaluated for each variable and the total number of participants in a particular
185 analysis was reported exactly. Analysis of the variables was ~~only performed~~ only if ~~when~~ the number
186 of participants was \geq at least 25% of the total cohort of male patients with CAH.

187 Three patients were excluded from part of the analyses as they received testosterone substitution,
188 which directly affects testosterone and gonadotropin concentrations. Two of these patients had data
189 on TART available; these are described in the results section, ~~but were otherwise excluded from~~
190 ~~further analyses.~~ Furthermore, we excluded 22 patients with missing genotype information and 2
191 patients with 11 β -hydroxylase deficiency from all comparative analyses.

192 **Results**

193 **General characteristics of the male CAH cohort**

194 A total of 121 male patients were included in the CAH cohort in the dsd-LIFE study. General
195 characteristics are shown in Table 1. The median age of the study population was 28 years (IQR: 18.5-
196 37.5, range 16-68). Mean height was 170.7 (95%CI: 169.3-172.0) cm and median BMI was 25.6 (IQR:
197 22.0-29.2) kg/m² (data available for 119 patients). **Nearly** ~~Almost~~ all patients had 21OHD (119/121),
198 ~~of which~~ **and** 97 were confirmed by molecular genetic analysis ~~and 22 were based on phenotype~~
199 ~~alone~~. The remaining 2 patients had 11 β -hydroxylase deficiency. **Among the 97 patients with**
200 **genetically confirmed 21OHD,** ~~Genotype groups null, A, B, and C contained 19.8% 24.7%~~ **were**
201 **classified as genotype null,** ~~30.6% 38.1%~~ **as genotype A,** ~~27.3% 34.0%~~ **as genotype B,** and ~~2.5% 3.1%~~
202 **as genotype C.** ~~of the 97 patients with genotyping results. The majority of patients (62.0%) were~~
203 ~~classified as having the SW form of CAH, 31.4% had the SV form and 4.1% had the NC form.~~
204 Glucocorticoids were used by 116 (95.9%) patients, most commonly hydrocortisone, followed by
205 prednisone or prednisolone, and dexamethasone. Fludrocortisone was used by 86 patients (71.1%).
206 The patients' education was intermediate or high in 54.5%, and 22.3% ~~of the participants,~~
207 respectively. Furthermore, 54.6% of the patients were in a relationship at the time of study.

208 **We analyzed all variables mentioned in the method section, but we only present in detail the data**
209 **that differed between the analyzed groups (no overlap in the confidence intervals). In the following**
210 **sections we will present data regarding hormone concentrations, semen analysis and TART.**

211 **Hormone concentrations**

212 **Univariate descriptive analyses of hormone concentrations were performed.** The proportion of
213 patients with normal, decreased or increased serum testosterone, LH, FSH, inhibin B, AMH, and SHBG
214 concentrations is illustrated in Figure 1A. Hormone concentrations were below the reference range
215 in 19/97 (19.6%: testosterone), 8/43 (18.6%: inhibin B), 12/90 (13.3%: LH), 9/90 (10.0%: FSH), and

216 1/69 (1.4%: SHBG) of the participants. SHBG concentrations were above the reference range in 14.5%
217 (10/69).

218 Table 2 shows compares testosterone and gonadotropin concentrations in all patients with data on T,
219 LH, and, FSH available. that in p Seven patients (50%) with decreased testosterone concentrations
220 (19/83), 8 (42.1%) had decreased gonadotropins, while 10 (52.6 42.9%) had normal LH and FSH
221 concentrations, and 1 (5.3 7.1%) patient had gonadotropin concentrations above reference range.
222 Normal testosterone concentrations were found in 64/83 55/69 (77.1 79.7%) patients, 50 44 (78.1
223 80.0%) of whom had normal gonadotropin concentrations, whereas 9 7 (14.1 12.7%) had increased,
224 and 5 4 (7.38%) had decreased concentrations. Decreased testosterone concentrations were clearly
225 associated with decreased LH and/or decreased FSH concentrations (OR 8.0 12.8, 95%CI: 2.9 - 2-
226 29.6 57.3).

227 A serum An AD/T ratio was calculated in 49 patients, 22 of whom (44.9%) had an AD/T ratio ≥ 1 . Ten
228 patients (45.5%) with an AD/T ≥ 1 had decreased gonadotropins, while 11 (50.0%) patients had
229 normal gonadotropins and only 1 (4.5%) patient had increased gonadotropins. Normal AD/T ratios
230 were found in 27/49 (55.1%) patients, 21 of whom had normal gonadotropin concentrations (77.8%),
231 5 had increased concentrations, but none had decreased gonadotropin concentrations. was found in
232 7/8 patients (87.5%) with decreased testosterone and gonadotropins, while 4/5 patients (80.0%)
233 with normal testosterone and decreased gonadotropins had an AD/T ratio ≥ 1 . Moreover, 5/10
234 patients (50.0%) with decreased testosterone and normal gonadotropins had an AD/T ratio ≥ 1 ,
235 whereas this was seen in only 11/50 patients (22.0%) with normal testosterone and gonadotropins.
236 An AD/T ratio ≥ 1 was strongly associated with decreased LH and/or decreased FSH concentrations
237 (OR 16.8 39.3, 95%CI: 2.10 - 142.5 732.4).

238 Semen analysis

239 Semen analysis was performed in approximately one third of the patients (Figure 1B). Normal values
240 for all known (at least 3 out of 5) semen parameters (normozoospermia) were seen in 11/39 patients

241 in which semen analysis was performed. Sperm concentration, motility, and volume were below the
242 normal ranges in 38.5% (15/39), 35.1% (13/37), and 25.6% (10/39) of the patients, respectively, while
243 morphology and vitality were both impaired in 14.3% (4/28 and 2/14) of the patients. Five of 8
244 patients (62.5%) with decreased testosterone and gonadotropin concentrations underwent semen
245 analysis, with 4 (80.0%) of them showing abnormal semen parameters (Table 3). In only 2/10
246 patients with decreased testosterone, but normal gonadotropin concentrations, semen analysis was
247 performed and both had decreased sperm concentrations (7.0 and 10.0 x10⁶/mL). No statistically
248 significant associations were found (data not shown).

249 **Testicular adrenal rest tumors**

250 TARTs were visualized by ultrasound or MRI at cross-sectional investigation in 28/68 patients. For 1
251 patient, the diagnosis was based on retrospective histology data. Furthermore, retrospective imaging
252 data were available for 11 men: TARTs were present in 10 of these individuals. So, in **11** the total
253 population screened, TARTs were present in 39/80 patients (48.8%) of which 34 were bilateral TARTs
254 (87.2%). Documented retrospective TARTs at age 16 years were reported in 16/30 patients (53.3%),
255 all of which were bilateral. In only 2/16 patients (12.5%) with TART reported to be present at age 16,
256 TART was no longer observed during the cross-sectional investigation: one patient was misdiagnosed
257 with TART as it appeared to be a varicocele, and in the other patient TART (size 2 mm) disappeared
258 after treatment with prednisone. This patient was still considered as a TART patient **with TART** in all
259 analyses.

260 *Comparison of patients with and without TART*

261 Table 4 shows associations of TART with various variables in the **78 68** patients with gonadal imaging
262 data (**12** patients were excluded due to testosterone substitution, **11 β -hydroxylase deficiency or**
263 **unconfirmed 21-hydroxylase deficiency**), comprising **37 33** patients with and **41 35** without TARTs.
264 Genotype was clearly associated with the presence of TART: The null genotype group had the highest
265 prevalence of TART (14/18: 77.8%), while the prevalence was 10/27 (37.0%) for genotype group A,

266 and 7/21 (33.3%) for genotype group B. The odds of having TART in the null genotype group was 6.0
267 [1.5-23.1] and 7.0 [1.7-29.4] times higher compared to the genotype groups A and B, respectively.
268 TARTs were also present in both men in the genotype C group, and also in 1 CYP11B1-deficient
269 patient (the other CYP11B1 patient did not undergo assessment for TART). The OR of having TART
270 when having an a serum androstenedione level concentration above the upper limit of normal at the
271 time of the cross-sectional investigation was 3.63 [1.0 - 11.2 12.7]. Similar associations were found
272 for serum 17OHP at the cross-sectional investigation, with an OR of 6.4 28.0 [1.7 3.1 - 24.7 252.5] for
273 having TART when 17OHP concentrations were more than twice the upper level of the reference
274 range, and an OR of 4 18.7 [1.3 2.2 - 158.1 16.5] when these concentrations were above the
275 reference range compared to concentrations within the reference range.

276 Paternity

277 Data on paternity were available for 113 of the 121 patients, 26 (23.0%) of whom (age range 26-68
278 years) had fathered a total of 43 children. Three couples had used assisted reproductive techniques
279 (ART) resulting in 4/43 children. One of the men who had used ART had decreased testosterone
280 concentrations, while another had increased FSH, decreased sperm concentration, and TART. No
281 information was available about the third patient who had used ART. ~~One man with impaired semen
282 motility, increased FSH concentrations, and TART had adopted a child.~~

283 Discussion

284 This unique and relatively large European multicenter study shows that gonadal dysfunction is a
285 common complication in male patients with CAH. Approximately half of the patients were affected
286 by endocrine disturbances of the HPG axis at an adult age and TARTs were present in approximately
287 half of the patients as well.

288 The difficulty in diagnosing hypogonadism in men with CAH is related to the fact that testosterone
289 measured in serum is a mixture of testosterone of gonadal and adrenal origin^{25,28}. Circulating

290 testosterone in male patients with well-controlled CAH is predominantly derived from testicular
291 production, but when there is poor hormonal control, a relevant contribution arises from adrenal
292 steroidogenesis. Until now, no method is able to discriminate between testosterone derived from
293 the testes or the adrenal gland. Therefore, it has been suggested to use the serum AD/T ratio in male
294 patients with CAH, as this precursor steroid is elevated in serum when serum androgens are
295 predominantly of adrenal origin²⁵. Our data point toward an association confirmed a stronger
296 association between an AD/T ratio ≥ 1 (testosterone mainly of adrenal origin) and decreased LH
297 and/or decreased FSH concentrations compared to testosterone concentrations alone, suggesting
298 that adrenal androgens in men with CAH contribute to the suppression of gonadotropins. In
299 approximately half of the patients, either aberrant testosterone or AD/T ratios, or aberrant
300 gonadotropin concentrations, or a combination of both were found. In previous studies, the reported
301 prevalence of endocrine HPG axis disturbances ranged from 20% to 52%^{5-7, 9, 10}. However, only 1
302 other report study provided had information on testosterone and gonadotropin concentrations in
303 each patient, and also indicated endocrine disturbances hypogonadism in approximately half of the
304 patients⁶. We recommend to including include the evaluation of the AD/T ratio in the regular follow-
305 up androstenedione measurements in the gonadal evaluation of male patients with CAH to calculate
306 the AD/T ratio, and interpret this ratio in combination with gonadotropin concentrations in order to
307 detect a disturbance of the HPG axis. Our study did does not include data information on 11-
308 oxygenated androgens, that are generated through conversion of androstenedione, and are reported
309 to be elevated concentrations are found in patients with CAH^{29, 30}. Recent studies indicate that 11-
310 oxygenated androgens are almost entirely derived from the 11beta-hydroxylation of
311 androstenedione in the adrenal, and as they are potent androgens they can contribute to
312 suppression of the HPG axis³¹. However, their exact role in the evaluation of However, their
313 associations with hormonal control and gonadal function in men with CAH has to be established in
314 further studies. Serum AMH and inhibin B are also used as markers for male fertility³². However,
315 literature already showed it has been demonstrated that serum AMH concentrations do not

316 correlate with sperm concentration and other male fertility parameters³³. Serum inhibin B, a marker
317 of Sertoli cell function, is known to correlate with spermatogenesis in healthy men³⁴ and was
318 decreased in 18.3% 18.6% of our cohort. Semen quality, assessed in one third of the study cohort,
319 was reduced in 40% of the men. Except for the study of Urban et al.³, all other studies on fertility in
320 male patients with CAH showed decreased sperm concentrations ranging from 47.8% to 66%^{4-7,9,10}.
321 More strikingly, in all studies only half of the participants participated in semen analysis. Taken
322 together, these data indicate the need for Therefore, increased awareness on fertility status in
323 patients with CAH, and to start is needed. We recommend performing semen analysis and gonadal
324 function biomarkers assessment as early as possible in from adolescence on, in order to detect
325 disturbances early and allow semen preservation to be able to preserve semen for later fertility
326 purposes.

327 Data from our cohort indicate, in agreement with previous studies The prevalence of TART in the
328 present patient cohort was 48.8%, confirming previous reports that TART is a common complication
329 in male patients with CAH^{4-10,12-22}, that TART is a common complication in males with CAH (with a
330 prevalence of 48.8%) and can have onset as early as in adolescence. In fact, Strikingly, 14 patients
331 with TART at the time of the dsd-LIFE study already had TART at the age of 16 years. TARTs
332 disappeared on at 16 years were no longer detectable following treatment with prednisone in only 1
333 patient, thus indicating. This could indicate that complete regression of TART might only be achieved
334 in a small proportion of the patients. Hence, prevention of the development of TART should be
335 pursued, by optimizing treatment strategies already in childhood. Current standard of care does not
336 include imaging of testes, however we recommend incorporating testicular ultrasound in routine
337 clinical practice.

338 In contrast to previous studies, several studies did not find an association between CAH severity and
339 TART^{4,9,10}, we observed an association between the CYP21A2 genotype and the presence of TARTs,
340 with the prevalence of this complication being was highest in men with the null CYP21A2 genotype.

341 This likely confirms supports the current perception that TARTs are more frequently observed in
342 patients with a more severe form of CAH, as these patients are exposed to higher concentrations of
343 ACTH, already *in utero*, which is thought to be a possible causative factor for TART development^{6, 7, 15,}
344²². However, a clinically relevant finding in this study is that TARTs occurs even in less severe forms of
345 21OHD. In fact, in ~~in~~ our study, 2 patients in genotype group C with NC CAH (both compound
346 heterozygous for deletion and P30L mutation) had TARTs. In our current dataset, we could not find
347 an association between genotype and semen quality or genotype and hypogonadism. Both patients
348 were compound heterozygous (deletion + P30L mutation). Only 1 patient in our cohort had the
349 typical NC mutation, i.e. the V281 mutation (V281/I2Splice). No TARTs were detected in this patient.
350 In our study, we We found an association between increased 17OHP concentrations at cross-
351 sectional data assessment and the presence of TART. Although a single 17OHP measurement may
352 not be representative of overall metabolic control, these results could be interpreted as a possible
353 indicator of the patient's metabolic control in the recent past. Therefore, our results seem to be in
354 accordance with literature reporting higher TART prevalence in patients with poor hormonal control
355 compared to patients with adequate hormonal control^{5, 7, 13, 35-38}. The association between increased
356 androstenedione concentrations at cross-sectional data assessment and the presence of TARTs adds
357 evidence to this pathophysiologic concept, even if the AD/T ratios were not clearly associated with
358 TART within this subgroup of patients. Primary gonadal dysfunction may be suggested by raised FSH
359 concentrations. In our dataset, 10 patients (11.1%) had elevated FSH concentrations. Seven of these
360 patients had data on the presence of TART, and 4 had evidence of TART. King et al. found that
361 testicular failure was a consequence of TART in the majority of cases¹⁰. However, our data are
362 limited and do not allow firm conclusions concerning this issue. We cannot confirm the findings of
363 King as we have only very limited data available.
364 Despite this being the first international multicenter study describing gonadal function in male
365 patients with CAH, the study also has some limitations. All centers included in this consortium are

366 tertiary care centers, therefore it is possible that the patient groups were selected and that the
367 patients included were more severely affected. Furthermore, serum hormone concentrations were
368 not measured centrally, but in various centers with a range of different assays. Accounting for this
369 fact, only range variables were used in the data analyses. The median BMI in our patient cohort was
370 25.6 kg/m² (range 22.0-29.2), which is slightly overweight. It has been demonstrated that excess of
371 total and abdominal body fat could represent one cause of fertility impairment in men with CAH²⁵.
372 Serum total testosterone can be decreased in patients with obesity, as a result of the decreased
373 serum concentration of SHBG. In case of increased serum SHBG (induced by hepatitis,
374 hyperthyroidism, or a genetic variant), total testosterone may be increased. Ideally, free testosterone
375 should be measured in these cases, but this requires complex equilibrium dialysis³⁹. Free
376 testosterone can also be calculated from the level of total testosterone, SHBG, and albumin
377 concentrations, but it is crucial that the results of such calculations are compared with the normal
378 range of each separate laboratory. Such data were not available. We are aware that assessment of
379 fertility by paternity numbers in our study was is incomplete, as many other factors, of which
380 including female fertility, are important as well. However, these data were not available.
381 Furthermore, participation in the medical examination was not obligatory compulsory for study
382 inclusion. This may have led to even more selection, especially concerning the ultrasound
383 examination and semen analysis. It is likely that only the very motivated patients and the more
384 severely affected patients consented to these additional examinations. Due to the resulting low
385 numbers of available data, multivariable logistic regression analyses were not possible.

386 In summary, impaired gonadal function is common in adult men with CAH. This is indicated by the
387 presence of TART and/or hypogonadotropic or hypergonadotropic hypogonadism. The risk of TART is
388 highest in men with the most severe forms of enzyme deficiencies underlying CAH. Our data suggest
389 that an association with poor previous hormonal control is likely but has to be confirmed requires
390 confirmation by further prospective studies. Determination of the serum AD/T ratio, in addition to
391 serum concentrations of testosterone, androstenedione, LH, and FSH may help to differentiate

392 between testicular and adrenal androgens in male patients with CAH and to estimate the degree
393 **diagnose** of gonadal dysfunction. Routinely performed semen analysis, measurement of serum
394 inhibin B, and testicular ultrasound investigation already in adolescence are recommended to detect
395 upcoming reproductive problems and to allow for fertility preserving measures, **such as sperm**
396 **banking.**

397

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404 **Declaration of interest**

405 The authors declare that there is no conflict of interest that could be perceived as prejudicing the
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539

540 **Figure legends**

541 **Figure 1: Hormone concentrations (A) and semen quality (B) in male patients with congenital**
542 **adrenal hyperplasia to assess gonadal function.** Stacked bars represent percentage of patients
543 within a category. Numbers in the bars represent the specific number of patients within a category,
544 while the total number of patients included in this analysis is stated underneath the x-axis. **A)**
545 Hormone concentrations of each patient were measured in the local hospital and compared to the
546 hospital's standard reference ranges. **B)** Semen analysis was performed and scored according to
547 World Health Organization 2010 criteria²⁶: sperm concentration, motility, morphology, and vitality,
548 and semen volume were assessed. Abbreviations: AMH, anti-Müllerian hormone; INHB, inhibin B; N,
549 number of patients; T, testosterone.