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PARACRINE REGULATION OF SPERMATOGENESIS BY GROWTH FACTORS
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We have established a testis tissue culture model which enables studies of the effects of growth factors on germ cell proliferation *in vitro*. Segments of seminiferous tubules of adult rat testes representing defined stages of the seminiferous epithelial cycle with representative premitotic and premeiotic germ cells were identified by transillumination and prepared by microdissection. The tubule segments were incubated at 34°and 37°C for 24, 48, and 72 h with and without growth factors and the DNA synthesis was determined at the end of the incubation. Recombinant epidermal growth factor (EGF) was found to stimulate premeiotic DNA synthesis in a dose-dependent fashion whereas premitotic DNA synthesis was only slightly affected. This indicates that EGF is mainly a meitotic growth factor during spermatogenesis. Together with previous results demonstrating specific effects of interleukin-1α, nerve growth factor and insulin-like growth factors, the present finding form the basis of a hypothesis that mitotic and meiotic proliferation of germ cells is differentially regulated by locally produced growth factors acting in a paracrine fashion.

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EFFECT OF ORAL TESTOSTERONE UNDECANOATE ON TANNER STAGE DEVELOPMENT GROWTH VELOCITY AND WEIGHT GAIN IN ANORCHID BOYS. H.Schmidt, D.Knorr, H.P.Schwarz, Department of Pediatrics, University of Munich, D-8000 Munich, Germany.

Ten patients with anorchia were treated with testosterone undecanoate (40 or 80mg/d) starting at the mean age of 11.5 years (range: 10 - 14 years). The effect on Tanner stage development, growth velocity and weight gain are presented. Therapy was started with 40mg 3x/week (n= 4), 40mg 7x/week (n=6), and was increased to 80mg 7x/week (n=9). Therapy was continued for 23.7 months (range: 14 - 40 months). Stretched penile length before therapy was $\bar{x}$ = 3.35cm (range: 2.5 - 4cm), at the end of therapy 6.25cm on average (range: 5 - 8cm). Pubic hair Tanner stage 2 (PH2) was achieved after 6.7 months (range: 4 - 14 months) PH3 after 20 months (range: 12 - 29 months). Growth velocity increased to 6.7cm/year (range: 3 - 9cm/year) in the first year of treatment, concomitant weight gain was 7.2kg (range: 5.2 - 10kg). No side effects of treatment were observed. We conclude that oral testosterone undecanoate therapy starting at the age of 11 years with 40mg/d for one year and 80mg/d for a second year is simple, safe and effective and provides for a gentle induction of puberty in anorchid boys.

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GONADAL FUNCTION AFTER FRACTIONATED TOTAL BODY IRRADIATION (TBI)
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Gonadal function was assessed in 28 children (12 male) treated with fractionated TBI (1200-1440 cGy) after conditioning cytotoxic chemotherapy (cyclophosphamide [n=26] or melphalan [n=2]). All had received prolonged cytotoxic chemotherapy before TBI. 3 boys had received direct testicular irradiation (600-1000 cGy). Gonadal function was assessed by measurement of basal sex steroids levels and the gonadotrophin response to GnRH. Leydig cell function was assessed by the testosterone (T) response to an intramuscular injection of HCG (1000 units). All 5 girls who were pubertal at the time of TBI developed ovarian failure (↑ gonadotrophins, ↓ oestradiol, exaggerated gonadotrophin responses to GnRH). The older prepubertal girls had ↑ basal gonadotrophins +/- exaggerated gonadotrophin responses to GnRH. All other prepubertal girls had normal prepubertal gonadotrophin levels. Unlike children who have received large radiation doses to the thorax, breast agenesis was not seen after induction of puberty with exogenous oestrogen. All boys were prepubertal at the time of TBI and five pubertal at assessment. Only one boy (prepubertal) had a normal T response to HCG. All 3 boys who had had direct testicular irradiation had abnormal T responses to HCG and elevated gonadotrophins. All other prepubertal boys had normal basal and stimulated gonadotrophin levels, but suboptimal T response to HCG. All pubertal boys at assessment had elevated gonadotrophins and suboptimal T response to HCG. Both boys in late puberty however had normal basal T levels. Testicular volumes were inappropriately small for pubertal stage. In conclusion the incidence of gonadal dysfunction is high after fractionated TBI. Ovarian failure is very common in the girls and severe damage to the germinal epithelium in boys is universal. Leydig cell damage is almost universal but the extent is often subtle. An HCG test can be helpful in determining the need for androgen replacement therapy which is difficult to predict.

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SHORT-TERM GROWTH AND ADULT HEIGHT IN CHILDREN AND ADOLESCENTS WITH 21-HYDROXYLASE DEFICIENCY: EFFECTS OF TREATMENT. B.P.Hauffa, A.Winter, H.Stolecke, Department of Pediatric Endocrinology, University of Essen, F.R.Germany

Adequate glucocorticoid and mineralocorticoid treatment is one of the prerequisites for normal growth in children with 21-hydroxylase deficiency. **Objectives:** To evaluate the effects of hydrocortisone dose, sex, salt-wasting status, and quality of treatment on short-term growth, and to compare adult height to target height. **Methods:** 569 growth episodes of 4-12 mo duration were analysed in 90 patients (females n=56; salt-wasting form n=42) with regard to height velocity SDS (HVSDS), bone age, and hydrocortisone dose. 37 patients had reached adult height. Quality of treatment during each episode was rated with the help of a score using clinical, laboratory and radiological criteria. **Results:** In girls with the salt-wasting variety (SW) a negative correlation of HVSDS for bone age ($HVSDS_{BA}$) with hydrocortisone dose was found (r= -0.41;p<0.006). If episodes with a HVSDS for chronological age ($HVSDS_{CA}$) outside the range of -2 -+2 were excluded, median daily hydrocortisone dose used in males (females) was 20.9 (20.6) mg/m²/d. Final height (m±SD) in girls (156.5±7.1 cm) and boys (169.2±10.0 cm) was 91-100.1% and 82.3-100% of target height with a trend towards taller adult heights in simple virilizing (SV) boys (90.3% vs. 96.5%; p<0.055). Stepwise multiple regression revealed an effect of quality of treatment on HVSDS in both prepubertal (p<0.0014) and pubertal (p<0.0016) subjects, and a minor effect of the salt-wasting status on $HVSDS_{CA}$ only in prepubertals (p<0.042). **Conclusions:** Although very high hydrocortisone doses were avoided in our patients, their mean adult height reaches only the 3. percentile of the normal population. Besides treatment quality and salt-wasting status there may be yet unknown factors involved in growth regulation of 21-hydroxylase deficiency patients.

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ADRENARCHE IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA (CAH) DUE TO 21-HYDROXYLASE DEFICIENCY. V. Brunelli, G. Chiumello, M. David and M.G. Forest, Department of Pediatrics and Endocrine unit, Scientific Institute H. S. Raffaele, University of Milan, Italy; Pediatric clinic and INSERM-U.329, Hôpital Debrousse, Lyon, France.

In order to evaluate adrenarche in female patients with CAH, plasma concentrations of dehydroepiandrosterone sulfate (DHEAS) were determined (by a specific RIA) longitudinally from 4 to 11 years of age in 16 CAH children in good clinical and biochemical control, presenting with either a severe classical form (group A; n = 10) or a non classical form (group B; n = 6). In group A, treatment was started at 5 ± 3.5 days of life [hydrocortisone initially 40 ± 5 mg/m2, spilt in 3 daily doses, lowered to 20 ± 5 mg/m2 as soon as a good control was obtained; fludrocortisone 37.5 µg/m2 per day initially, then at maintenance dose of 25 µg/m2/day]. Therapy was started at a mean age of 5.7 ± 0.9 years in group B [hydrocortisone 15 ± 5 mg/m2 in 3 daily doses]. Results (in nmol/L) are given as mean ± SD in table 1. In group A, DHAS levels were very low at any age, lower than in controls but also lower than in group B. In the latter, DHAS levels were initially very high for age, then decreased slowly under treatment. In both groups there was no significant rise of DHEAS after age 7-9.

Table 1.	< 6 years	6-7 years	7-9 years	9-11 years
Group A	19 ± 14[bd]	34 ± 26[bd]	31± 29[bd]	56 ± 58[bd]
Group B	1855 ± 1086[cd]	401 ± 327[c]	213 ± 62[cd]	261 ± 150[cd]
Controls	59 ± 40	207 ± 174[a]	742 ± 384[a]	1166 ± 435[a]

p<0.05 vs previous age group = a; vs same age in groups B (= b), A (= c), or controls (= d)

In conclusion : 1). Despite good clinical and biochemical control in these 16 selected patients treated with conventional therapy, a physiological adrenarche didn't occur whatever the age of the onset of the disease. 2). The lack of adrenarche is not due to a decreased capacity to synthesize DHAS, since its levels were very high in untreated girls with non classical CAH. 3). The mechanism by which DHEAS secretion is impaired under substitutive therapy at physiological dose is still not well understood.

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RELATIONSHIP BETWEEN GENOTYPE AND PHENOTYPE IN GERMAN FAMILIES WITH CONGENITAL ADRENAL HYPERPLASIA (CAH) DUE TO 21 HYDROXYLASE DEFICIENCY A. Serban*#, D. L'Allemand#, H. Helge#, M. Murena*, Y. Morel*. *Inserm U329, Hôpital Debrousse, 69322 Lyon, Cedex05, France. #Dept. of Pediatrics, Free University, 1000 Berlin 19, Germany

In 13 unrelated CAH families (2 with 2 patients) from Berlin, Germany, the CYP21B gene and its region were studied to establish if the incidence of each genetic lesion and the relationship between genotype and phenotype are similar to these of other populations. Among our families, 6 had SW, 5 SV and 2 NC form. 26 different CAH haplotypes have been analyzed using the simple strategy combining Southern blotting studies and specific amplification of the CYP21B gene by PCR as previously described (*Endocri Soc 1991, abstr. 1379*). To-date screening for 8 point mutations (PM) of the CYP21B gene was done. The most common lesions were large deletions (6/26, 23%) and two PM: 668-13C→G (6/26, 23%) and I172N (5/26, 20%). Less frequent were large gene conversions (2/26, 8%) and PM G318X (1/26, 4%) and V281L (2/26, 8%). On 15% (4/26) of the haplotypes, no mutation could be found yet. A good correlation exists between genotype and phenotype. All patients homozygous or double heterozygous for large deletions, gene conversions or G318X had a SW form. I172N was correlated with SV form in homozygous as well as in double heterozygous patients. V281L was found in 1 patient with NC form. In contrast, 668-13C→G mutation did not correlate well with clinical expression: two homozygous patients for 668-13C→G had neonatal SW crisis, but actually, endocrine investigation shows no more SW, even without treatment. HLA typing confirmed that HLA-Bw47 (2) is associated with CYP21B large deletion; HLA Bw51, often associated with SV form in german population, was found with different genetic lesions and all phenotypes in our patients. Almost all families (11/13) were fully informative for prenatal diagnosis when determining the PM in association with our Southern appproach; the 2 remaining ones (1SV, 1NC) were semi-informative. This direct molecular study of the CYP21B gene could be used for prenatal diagnosis.