International Journal of Andrology
international journal of andrology ISSN 0105-6263

ORIGINAL ARTICLE

The hypothalamus-pituitary-testis axis in boys during the first six months of life: a comparison of cryptorchidism and hypospadias cases with controls

Frank H. Pierik,*† James A. Deddens,‡ Alex Burdorf,† Sabine M. P. F. de Muinck Keizer-Schrama,§ Frank H. de Jong¶ and Rob F. A. Weber*

*Department of Andrology, Erasmus MC, †Department of Public Health, Erasmus MC, Rotterdam, The Netherlands, ‡National Institute for Occupational Safety and Health, Cincinnati, OH, USA, §Department of Pediatric Endocrinology, Erasmus MC, and ¶Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands

Keywords:
AHM, cryptorchidism, FSH, hypospadias, inhibin B, LH, newborn boys, testosterone

Correspondence:
Frank H. Pierik, TNO Environment, Health and Safety, Department of Environment and Health, PO Box 49, 2600 AA Delft, The Netherlands.
E-mail: frank.pierik@tno.nl

Received 13 July 2007; revised 12 January 2008; accepted 16 January 2008

doi:10.1111/j.1365-2605.2008.00877.x

Summary

It is inconclusive whether the feedback mechanisms of the hypothalamus-pituitary-testis (HTP) axis are already established in the first 6 months of life, partly due to the dramatic changes in HPT-axis hormone levels over this period. Moreover, it is unclear whether these hormone levels are aberrant in boys with cryptorchidism or hypospadias, and therefore predictive for future fertility. We studied the regulation mechanisms of the HTP axis, and the effect of age, in boys 1–6 months of age. Secondly, we studied testicular function - as reflected by HPT hormones - in newborns with cryptorchidism or hypospadias. Sera from a population sample of infants with cryptorchidism (n = 43), hypospadias (n = 41) and controls (n = 113) were analyzed for inhibin B, anti-Müllerian hormone (AMH), testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and sex hormone binding globulin (SHBG). LH, testosterone, non-shbg-bound testosterone (NSBT), and AHM levels showed significant age-related trends. After age-correction, a negative correlation between FSH and inhibin B was observed (r = −0.43). The only significant group-differences were lower testosterone and NSBT levels in cryptorchidism cases, with a mean testosterone of 1.8 and 2.6 nmol/L and a mean NSBT of 0.48 and 0.70 nmol/L for cryptorchidism cases and controls, respectively. The higher levels of LH, testosterone, and NSBT in boys born pre-term or with a low birthweight indicate that abnormal prenatal development may determine postnatal testis function. Our results support the hypothesis that the inhibin B – FSH feedback loop is already functional before puberty. The lower testosterone and NSBT levels indicate that disturbed Leydig cell function can already be detected early after birth in cryptorchid boys.

Introduction

Little is known about the regulation of the secretion of the hormones in the hypothalamus-pituitary-testis (HPT) axis during the first 6 months of life, and its relevance for testis function in adulthood. In adult men, gonadotropins are under negative feedback control of the gonadal androgens and inhibin B (Anderson & Sharpe, 2000; Andersson, 2000). It is, however, unclear when these feedback loops are established (i.e. prenatally, at birth, in childhood, or during puberty) (Anderson & Sharpe, 2000; Andersson, 2000; Pierik *et al.*, 2003). The literature is contradictory with respect to the regulation mechanisms that control the HPT axis before puberty. Some studies show that the negative feedback actions of testosterone on LH and of inhibin B on FSH are already functional in boys during infancy whereas other data suggests a positive or no relationship (Andersson *et al.*, 1997; Crofton *et al.*, 1997, 2002; Chada *et al.*, 2003). This discrepancy may be due to the fact that these hormone levels are highly age-dependent in postnatal life. Moreover, the age at examination was not identical in these studies.

In the last decade, anti-Müllerian hormone (AMH) and inhibin B have been described as testicular hormones that can be detected throughout life, whereas other HPT hormones are at very low or undetectable levels between 6 months of age and the onset of puberty (Anderson & Sharpe, 2000; Rey, 2000; Andersson & Skakkebaek, 2001). Both AMH and inhibin B are Sertoli cell products, and serum levels may reflect the number of Sertoli cells or their function. Each Sertoli cell can support a limited number of germ cells during spermatogenesis, and the number of Sertoli cells in the testes probably limits the capacity to produce spermatozoa in adulthood (Sharpe *et al.*, 2003). Therefore, AMH and inhibin B measured in newborns may predict future male fertility (Pierik *et al.*, 2003) which may be impaired in cases of cryptorchidism and hypospadias.

About 2–5% of newborn boys are diagnosed with cryptorchidism or hypospadias (Toppari *et al.*, 2001). There is strong evidence that men affected by cryptorchidism in early life are at increased risk for male infertility, reduced sperm quality and testicular germ-cell tumors (Swerdlow *et al.*, 1997; Lee & Coughlin, 2001). Hypospadias is more frequent among sons of subfertile men, and may be more frequently present among cases of cryptorchidism (John Radcliffe Hospital Cryptorchidism Study Group, 1992). Testicular function may be impaired among cases of hypospadias (Skakkebæk *et al.*, 2001). Although abnormal hormonal profiles have been observed in adult cryptorchidism cases (de Gouveia Brazao *et al.*, 2003), only a few studies have published findings on hormone levels before puberty (Barthold *et al.*, 2004; Suomi *et al.*, 2006; Toppari *et al.*, 2007).

The objective of our study was to assess whether HPT-axis feedback-loops that operate in adulthood are already in place in early life. The second objective was to study HPT hormones in neonatal boys with cryptorchidism and hypospadias.

Materials and methods

Population and design

To study HPT-hormones in boys with hypospadias or cryptorchidism and controls, we asked the parents of the boys in a case-control study on risk factors for both abnormalities permission to obtain a serum sample from the boys. Given the aim to study the HPT axis in the first months of life, around the postnatal surge at 3–4 months of age, we only collected blood samples in boys less than 6 months of age. The case-control study has been described in detail elsewhere (Pierik *et al.*, 2004), and is shortly specified below. It was nested within a large cohort of newborn boys in the city of Rotterdam, who were examined at their first visit to Child Healthcare Centers (CHCs). CHCs invite all parents to participate free of charge in the nationwide preventive child healthcare program, including vaccinations. In the period October 1999 through December 2001, 9146 male births were registered of which 8695 boys (95%) were examined by CHC physicians at a median age of 34 days (5th, 95th percentiles were 25, 105 days). CHC physicians ($n = 30$) were trained to perform a standardized examination for cryptorchidism and hypospadias (Pierik *et al.*, 2002, 2005). In the original case-control study, 78 cryptorchidism cases, 56 hypospadias cases and 313 control subjects were studied. For the purpose of the present study, blood collection was successful (defined as at least 3 mL of blood) in 43/78 cryptorchidism cases, 41/56 hypospadias cases, and 113/313 boys without either abnormality. The age range at blood collection (minimum, maximum) for these groups were 40–184 days, 35–171 days, and 39–155 days, respectively.

The main reasons for not having blood for all cryptorchidism and hypospadias cases were: we did not collect blood when boys were above 6 months of age, and in several cases blood collection was unsuccessful or yielded an insufficient volume of serum. We discontinued blood collection among controls when at least one control sample per case was available, which explains the lower proportion of blood collection among the controls in the original study. The institutional review board has approved the study protocol.

Questionnaire

A research nurse completed a structured questionnaire during interviews with the mothers to assess risk factors for cryptorchidism and hypospadias as described previously (Pierik *et al.*, 2004). Since these risk factors may also be of relevance for HPT hormones in the children, they were included in the present study. The questionnaire focused on personal characteristics, health, and pregnancy aspects. Personal characteristics were age, length, weight, education, country of origin, and lifestyle factors. Information was also collected on time-to-pregnancy (in months), parity, weeks of gestation, birthweight, and whether the pregnancy was induced by assisted reproduction technologies (ART). Babies were defined as small for gestational age (SGA) when their birthweight was more than two standard deviations below the reference value for their gestational age (Usher & McLean, 1969). Pre-term delivery was defined as a duration of gestation of 37 weeks or less.

Hormone analysis

Serum samples were stored at −20 °C and analyzed in two batches. Inhibin B was measured using kits purchased

from Serotec Ltd, Oxford, UK. Within-assay and between-assay coefficient of variation (CV) were less than 9%, and less than 15%, respectively. Serum FSH and LH were determined with the Immulite assay (Diagnostic Products Corporation, Los Angeles, USA). Within-assay and between-assay CV were less than 6 and 9%; and less than 5 and 11% for FSH and LH, respectively. Sex hormone binding globulin (SHBG) was also determined using an Immulite assay (within and between-assay CV: <4 and <7%). An ultra-sensitive immuno-enzymometric assay (Immunotech-Coulter, Marseille, France) was used for the estimation of AMH. Within- and between-assay coefficients of variation were <5 and <8%, respectively. The non-SHBG-bound testosterone level was calculated using the method described by Sodergard *et al.* (1982) using a fixed serum albumin concentration of 40 g/L.

Total serum testosterone was determined by radioimmunoassay using coated tubes obtained from the same supplier (within- and between-assay CV: <6 and <9%). Although some problems have been described when extraction assay results are compared with data obtained using a direct method (Tomlinson *et al.*, 2004), after day 10 of life the results of both types of assays agree well with our results for normal boys.

The detection limits for the assays were 0.14 nmol/L for testosterone, 0.1 IU/L for LH, 0.1 IU/L for FSH, 0.2 nmol/L for SHBG, 10 ng/L for inhibin B, and 0.05 μg/L for AMH. Levels that were below the limit of detection were assigned the detection limit divided by 2.

Statistical methods

All hormones levels were log-transformed to satisfy the normal distribution assumption. An analysis of the relationship of hormone level to age at blood collection was undertaken. In order to model the shape of this relationship, we used restricted cubic splines (Harrell *et al.*, 1988). Given a certain number of knots, a restricted cubic spline is a smooth curve which is cubic between the knots, smooth at the knots, and linear at both ends. Considering only children in the control group we tested various choices for the number of knots. Testosterone and NSBT had significant non-linear relationships with the age at blood collection, and a 3-knot restricted cubic spline provided a good fit. For simplicity of presentation we included a 3-knot restricted cubic spline in analyses of all hormones, even when the relationship with age was linear.

Firstly, the relationships between the hormones and age were plotted for presentation. Secondly, in a univariate analysis, we compared the general demographic and hormone characteristics between groups of boys (controls, cryptorchidism, hypospadias). Thirdly, to study HPT feedback mechanisms, we computed the partial correlation coefficients between the log-transformed hormone levels with adjustment for age at blood collection using a restricted cubic spline. Fourthly, considering only the children in the control group and treating the log-transformed hormone level as the dependent variables we examined which covariates in normal boys were associated with the HPT-axis hormones under study; hormone analysis batch and the restricted cubic spline of age at blood collection were included in this model. Adjusted means with 95% confidence intervals were computed. Finally, using all children we studied all log-transformed hormone levels as the dependent variables with case status (the groups of cryptorchidism, hypospadias and controls), hormone analysis batch and a restricted cubic spline in the model, and we performed a stepwise regression analysis of all other covariates to estimate the adjusted means for cryptorchidism, hypospadias, and control groups. The covariates under study were birthweight, gestational age, small for gestational age, pre-term delivery, primary parity, assisted reproduction, age of the mother, mothers health, height, weight, educational level, and ethnic origin. All models were adjusted for covariates that were significant in any of the final stepwise regression models per hormone. Interaction between the covariates and disease group was also assessed.

Results

Figure 1 shows the relationship between the levels of HPT hormones and the age of the newborn at the time of blood collection among boys without cryptorchidism and hypospadias. The serum testosterone, LH level and NSBT levels were significantly negatively associated with the age at blood collection ($p < 0.05$), AMH was positively associated with age ($p < 0.05$), whereas serum SHBG, inhibin B, and FSH were not significantly associated with age at blood collection. We considered the interaction of the 3-knot restricted cubic spline with disease group and found it to be non-significant for all hormones.

Table 1 gives general characteristics for the boys with cryptorchidism or hypospadias and controls. Pre-term birth, birthweight, Turkish origin, the age at blood collection, and the serum levels of testosterone and NSBT (both unadjusted for age at blood collection) were significantly different between groups when tested in a univariate analysis. Table 2 presents partial correlations between the individual log transformed hormone levels in newborns without cryptorchidism and hypospadias, corrected for the age at blood collection. Negative associations were statistically significant for FSH vs. inhibin B, FSH vs. AMH, and positive associations were observed for testosterone vs. SHBG, NSBT, and LH, for SHBG vs. LH,

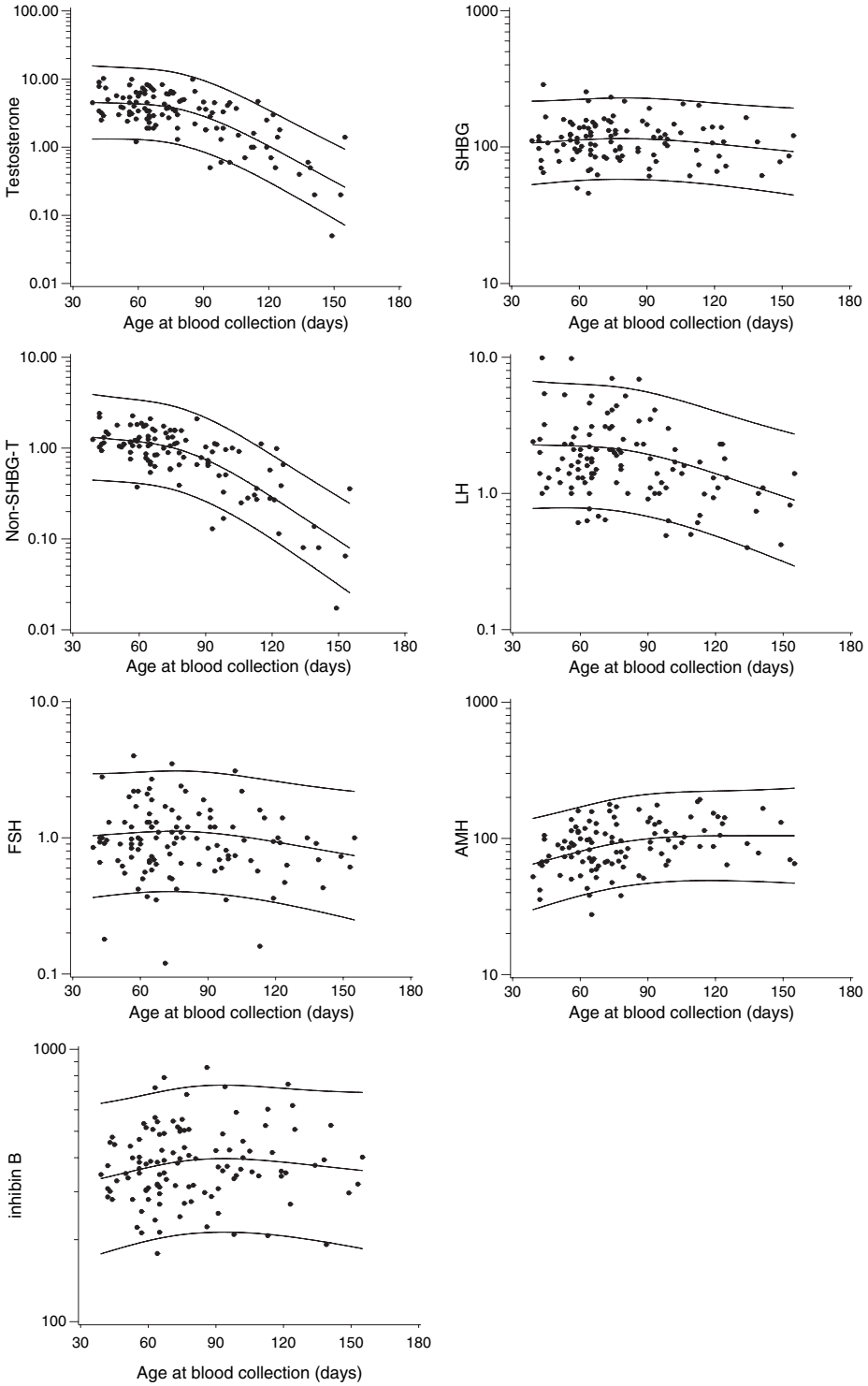

Figure 1 Relation between HPT hormone levels and the age at blood collection for the reference population. The plotted line is based on the fitted spline function, with 95% prediction intervals. SHBG, inhibin B and FSH were not significantly related to the age at blood collection. Hormone levels are in the following units: nmol/L for testosterone, SHBG, and non-SHBG-bound testosterone, ng/L for inhibin B, μg/L for AMH, IU/L for FSH and LH. In the legend it should be noted that there is not a significant age relationship for SHBG, inhibin B and FSH.

Table 1 Comparison of the characteristics and hormone levels between subgroups of normal boys and boys with cryptorchidism or hypospadias (total *n* = 197); mean (standard deviation) or proportion of the subgroup (percentage)

Variable	Controls (*n* = 113)	(SD or %)	Cryptorchidism (*n* = 43)	(SD or %)	Hypospadias (*n* = 41)	(SD or %)
Birthweight (grams)	3410	(507)	3347	(555)	3111**	(581)
SGA	5/110	(5%)	1/42	(2%)	5/40	(13%)
Preterm birth	5/112	(4%)	8/43*	(19%)	8/41*	(20%)
Firstborn child	53/112	(47%)	26/43	(60%)	17/41	(41%)
Maternal age (years)	30.8	(5.2)	29.2	(6.0)	30.1	(5.1)
Country of origin mother						
Netherlands	51/112	(46%)	17/43	(40%)	20/41	(49%)
Morocco	9/112	(8%)	3/43	(7%)	1/41	(2%)
Turkey	4/112	(4%)	8/43*	(19%)	7/41*	(17%)
Surinam	12/112	(11%)	6/43	(14%)	3/41	(7%)
Other	36/112	(32%)	9/43	(21%)	10/41	(24%)
Suboptimal maternal health	11/112	(10%)	7/43	(16%)	8/41	(20%)
Age at blood draw (days)	80	(27)	85	(31)	94**	(40)
Testosterone[a] (nmol/L)	2.8	(2.5)	1.8**	(4.3)	1.8**	(4.2)
SHBG[a] (nmol/L)	110.9	(1.4)	109.8	(1.4)	107.4	(1.5)
NSBT (nmol/L)	0.8	(2.5)	0.5*	(4.3)	0.5*	(4.2)
LH[a] (IU/L)	1.9	(1.8)	2.1	(2.0)	1.5	(1.8)
FSH[a] (IU/L)	1.1	(1.7)	1.2	(1.8)	1.0	(1.8)
AMH[a] (μg/L)	88.4	(1.5)	87.2	(1.5)	87.0	(1.5)
Inhibin B[a] (ng/L)	376.6	(1.4)	371.6	(1.3)	354.1	(1.3)

SGA: being born small-for-gestational age (mean – 2 SD, see Methods).
[a]Means are geometric means with geometric standard deviations.
*$p < 0.05$ vs. control group (Chi-square).
**$p < 0.05$ vs. control group (ANOVA).

Table 2 Correlations between log-transformed serum levels of hypothalamus-pituitary-testis axis hormones in 110 newborn boys from the general population (excluding cryptorchidism or hypospadias), adjusted for age at blood collection and analysis batch

	SHBG	NSBT	LH	FSH	AMH	Inhibin B
T	**0.48**	**0.91**	**0.31**	0.02	0.001	0.14
	p < 0.001	**p < 0.001**	**p = 0.001**	*p* = 0.85	*p* = 0.99	*p* = 0.14
SHBG		0.07	**0.20**	−0.02	−0.03	0.03
		p = 0.50	**p = 0.04**	*p* = 0.84	*p* = 0.74	*p* = 0.73
NSBT			**0.27**	0.09	0.01	0.14
			p = 0.006	*p* = 0.35	*p* = 0.94	*p* = 0.16
LH				**0.28**	−0.11	−0.04
				p = 0.003	*p* = 0.27	*p* = 0.72
FSH					**−0.28**	**−0.43**
					p = 0.003	**p < 0.001**
AMH						**0.42**
						p < 0.001

T, testosterone; SHBG, sex-hormone binding globulin; NSBT, non-SHBG-bound testosterone.

for NSBT vs. LH, for LH vs. FSH, and for AMH vs. inhibin B.

Table 3 shows the covariates that were significantly associated with the serum level of any of the studied HPT-hormones in normal boys. Several of these covariates were interrelated (i.e. birthweight, pre-term birth, being born small-for-gestational-age). In general, a reduced intrauterine growth – as indicated by low birthweight or pre-term delivery - was associated with a higher LH, higher NSBT, and a higher testosterone. Specifically, SHBG and NSBT were higher for pre-term infants, and LH was higher for boys born small for gestational age. For SHBG there was no clear direction of the effect, since it was higher among boys with a birthweight less than 3000 g, and lower for pre-mature births. Besides pregnancy related covariates, some of the hormones were also significantly associated with the country of origin of the mother (lower LH and FSH for children with Turkish mother), and in boys born after assisted reproduction, the NSBT and inhibin B levels were lower

Table 3 Covariates associated with serum levels of LH, FSH, testosterone, AMH, SHBG, NSBT, and inhibin B in 110 boys without cryptorchidism and hypospadias, after adjustment for age of blood draw and batch differences (geometric means at the average age of 80 days with, between brackets, the 95% confidence interval)

	Testosterone	SHBG	NSBT	LH	FSH	AMH	Inhibin B
Birthweight (grams)							
<3000	3.6 (2.8–4.6)**	130 (112–150)**	0.92 (0.74–1.14)	2.4 (1.9–2.9)*	1.1 (0.9–1.4)	80 (69–93)	374 (331–423)
≥3000	2.7 (2.4–3.1)	107 (99–115)	0.75 (0.67–0.84)	1.9 (1.7–2.1)	1.0 (0.9–1.1)	91 (84–99)	380 (356–404)
Premature birth							
Yes	4.0 (2.3–7.0)	84 (62–115)*	1.32 (0.83–2.11)**	1.7 (1.1–2.8)	1.1 (0.7–1.7)	101 (72–141)	423 (325–551)
No	2.8 (2.5–3.1)	113 (105–121)	0.76 (0.69–0.84)	1.9 (1.8–2.2)	1.0 (1.0–1.2)	88 (81–94)	375 (354–397)
Small-for-gest. age							
Yes	2.9 (1.7–5.1)	104 (76–142)	0.84 (0.52–1.35)	3.7 (2.4–5.9)**	1.2 (0.7–1.9)	69 (50–97)	322 (248–419)
No	2.9 (2.6–3.2)	112 (104–120)	0.78 (0.70–0.86)	1.9 (1.7–2.1)	1.0 (0.9–1.2)	89 (83–96)	381 (361–404)
Firstborn							
Yes	3.1 (2.6–3.6)	115 (104–126)	0.82 (0.70–0.95)	1.9 (1.7–2.2)	1.0 (0.8–1.1)*	89 (81–101)	394 (364–427)
No	2.6 (2.3–3.1)	108 (98–119)	0.75 (0.65–0.87)	1.9 (1.7–2.2)	1.2 (1.0–1.3)	87 (79–95)	362 (335–391)
Assisted reproduction techniques							
Yes	2.8 (2.4–3.1)	111 (104–119)	0.77 (0.69–0.85)*	1.9 (1.7–2.1)	1.1 (1.0–1.2)	88 (82–95)	373 (353–394)**
No	4.9 (2.4–9.9)	106 (70–160)	1.43 (0.77–2.66)	2.8 (1.5–5.3)	0.7 (0.4–1.3)	93 (60–146)	546 (387–769)
Good general health mother							
Yes	2.8 (2.4–3.1)	109 (102–117)	0.79 (0.57–1.11)	1.9 (1.7–2.1)	1.0 (0.9–1.1)	88 (82–95)	379 (357–402)
No	3.5 (2.5–5.1)	131 (106–163)	0.78 (0.70–0.87)	2.3 (1.8–3.3)	1.4 (1.0–1.9)*	89 (70–112)	359 (301–429)
Maternal origin							
Turkish	2.9 (1.6–5.2)	91 (60–136)	0.90 (0.49–1.68)	1.2 (0.7–2.1)*	0.6 (0.4–1.0)**	88 (57–136)	463 (346–622)
Other	2.8 (2.5–3.2)	112 (105–120)	0.78 (0.70–0.86)	2.0 (1.8–2.2)	1.1 (1.0–1.2)	88 (82–95)	374 (354–396)
Soy-protein intake							
≥20 g/day	3.6 (2.6–5.0)	139 (114–170)**	0.89 (0.65–1.22)	2.2 (1.6–2.9)	1.2 (0.9–1.6)	88 (72–109)	354 (302–416)
<20 g/day	2.7 (2.4–3.1)	108 (101–115)	0.78 (0.69–0.86)	1.9 (1.7–2.1)	1.0 (0.9–1.1)	88 (82–95)	380 (358–403)

*$p < 0.10$; **$p < 0.05$.

and higher, respectively, than in boys conceived spontaneously.

Table 4 gives the adjusted geometric mean hormone levels with 95% confidence intervals for controls and for boys with cryptorchidism and hypospadias. These levels were adjusted for hormone analysis batch, age at blood collection, being small for gestational age, maternal health status, pre-term delivery, primary parity, Turkish origin and assisted reproductive techniques. The only significant differences were the lower level of testosterone and NSBT in cryptorchidism cases, with a mean testosterone of 1.8 and 2.5 nmol/L and a mean NSBT of 0.48 and 0.70 nmol/L for cryptorchidism cases and controls, respectively. Compared with controls, a higher proportion of cryptorchidism cases had a testosterone level below the limit of detection (3/43 vs. 1/113, Fisher exact test p-value = 0.06, and 4/41 vs. 1/113, Fisher exact test p-value = 0.02).

Table 4 Geometric serum levels of hypothalamus-pituitary-testis axis hormones, adjusted for age, being small for gestational age, maternal health status, pre-term delivery, primary parity and Turkish origin (geometric means and 95% confidence intervals, at the average age of 84 days)

	Controls (n = 110)		Cryptorchidism (n = 43)		Hypospadias (n = 41)	
Hormone	adj GM	95% CI	adj GM	95% CI	adj GM	95% CI
Testosterone	2.5	(2.2–3.0)	1.8*	(1.4–2.3)	2.4	(1.9–3.2)
SHBG	108.4	(101.3–116.3)	110.9	(98.3–125.3)	112.4	(99.3–127.3)
NSBT	0.70	(0.61–0.82)	0.48**	(0.37–0.62)	0.65	(0.50–0.85)
LH	1.9	(1.7–2.1)	2.1	(1.8–2.5)	1.6	(1.4–1.9)
FSH	1.0	(0.9–1.1)	1.2	(1.0–1.4)	1.0	(0.8–1.2)
AMH	89.1	(82.6–95.9)	85.7	(75.4–97.5)	87.6	(77.2–99.4)
Inhibin B	377.0	(357.5–397.6)	369.7	(338.6–403.6)	365.7	(333.6–400.8)

adj. GM, adjusted geometric mean; 95% CI, 95% confidence intervals around adj. GM.
*p = 0.03 compared with controls, **p = 0.01 compared with controls.

Discussion

The negative feedback of inhibin B on FSH levels is presumably already established in the infant boy although the evidence is not fully conclusive (discussed by Andersson & Skakkebaek, 2001). A significant negative correlation between inhibin B and FSH has not been consistently observed. Previous papers reported the absence (Andersson *et al.*, 1998) or presence (Raivio & Dunkel, 1999) of a negative relationship between the serum levels of these peptide hormones in boys of 0–2 years of age, or even a positive relation (Chada *et al.*, 2003). This discrepancy might be due to the dramatic hormonal changes that take place in the first months of life, when the activity of the HPT axis is rapidly increasing and then decreasing (Andersson *et al.*, 1998). By adjusting for the time since birth, we indirectly attempted to adjust for this postnatal peak of activation of the pituitary-gonadal axis, and observed a negative relation between FSH and inhibin B results. Because our data are based on a single baseline measurement per individual, our results are only indirect support for the hypothesis that a dynamic negative feedback is already in place in boys of 1–3 months of age.

The AMH level is probably a marker of Sertoli cell number and function, but it is unclear what its physiological role is after prenatal sex-differentiation (Fenichel *et al.*, 1999). The negative association with FSH could well be the result of the negative feedback by inhibin B which is produced concomitantly with AMH by Sertoli cells, as reflected by the strong positive correlation between AMH and inhibin B levels. The positive association of testosterone with SHBG and NSBT and the lack of correlation between SHBG and NSBT, has been discussed previously (de Ronde *et al.*, 2005), and the positive relation between LH vs. FSH is comparable to the regulation mechanisms in adult males (Rosner, 1990; Pierik *et al.*, 1998; de Ronde *et al.*, 2005). A positive correlation between LH and testosterone in newborns and children has been reported before (Gendrel *et al.*, 1980; Chada *et al.*, 2003), and does not provide evidence for a negative feedback of testosterone on LH already in the first 6 months of life.

We studied the relation between HPT hormone levels and age, and observed an age-dependency for serum concentrations of testosterone, LH, AMH, and NSBT in boys in the first 6 months of life. The age-related decline was expected based on previous studies on HPT hormone levels throughout childhood (Andersson *et al.*, 1998; Andersson & Skakkebaek, 2001; Crofton *et al.*, 2002; Raivio & Dunkel, 2002). We did not observe a trend with age in the serum levels of SHBG, inhibin B and FSH, which may be explained by the fact that we studied only a single sample per individual, in a short time-window. Repeated longitudinal samples are needed to further establish age-related trends during child development. Previous studies over a longer period of childhood do also describe a decline in inhibin B, SHBG and FSH, but slower than for testosterone (Andersson *et al.*, 1998). The age-dependency illustrated the need for age-adjustment in further analyses, i.e. when comparing subgroups or when studying correlations between HPT hormones. The data on the subjects without cryptorchidism and hypospadias provide additional reference data for the levels of specified HPT hormones in early childhood (Chada *et al.*, 2003; Bergada *et al.*, 2006).

This study demonstrates a lower basal serum total testosterone level and a lower free androgen level (NSBT) in male infants diagnosed with cryptorchidism, but not in cases of hypospadias, with statistical adjustments for age, being small for gestational age, pre-term delivery, maternal health, primary parity and Turkish origin. Statistical adjustment for SGA and pre-term delivery may be inappropriate if these factors play a role within the causal pathway. However, omission of these variables from the model did not change the effects. To test whether the ethnic heterogeneity biased our results we ran the final models on the subjects of Dutch origin only, yielding identical conclusions (data not shown).

Previous studies also reported lower androgen levels in cryptorchidism cases (Gendrel *et al.*, 1980; Raivio *et al.*, 2003) or a blunted postnatal rise in testosterone in some cryptorchid babies (Gendrel *et al.*, 1980). In two more recent studies, the androgens levels were not different between cryptorchid and normal boys that were assessed before or around the age of 3 months (Barthold *et al.*, 2004; Suomi *et al.*, 2006). The latter of these studies, however, observed lower androgen levels in the subgroup of boys with severe and persistent cryptorchidism (Suomi *et al.*, 2006). The severity of cryptorchidism, which may explain the low NSBT levels we observed, was unfortunately not known for the majority of cases in our study.

We observed that the lower testosterone and NSBT was mainly due to a higher proportion of testosterone levels below the detection limit among boys with cryptorchidism that were 100 or more days old, suggesting that in cryptorchidism cases the androgen nadir may be reached more rapidly. Several studies have reported that testosterone levels fall to very low levels, even under the limit of detection, at 3–6 months of age (Andersson *et al.*, 1998; Raivio & Dunkel, 1999; Pierik *et al.*, 2003).

Previous studies have suggested serum AMH and inhibin B levels as markers of the number of Sertoli cells in infancy, hence reflecting the spermatogenetic capacity in adulthood. We did not observe differences in AMH and inhibin B levels between boys with cryptorchidism, hypospadias and controls. This may indicate that the

number and function of Sertoli cells is affected in boys with these urogenital abnormalities at a later age, or that such damage is not yet reflected by sex-hormone levels at this age.

We also studied whether some of the previously studied risk factors for cryptorchidism and hypospadias (Pierik *et al.*, 2004) were associated with the HPT hormone levels. Some of the variables related to intrauterine growth (low birthweight, small-for-gestational age, pre-mature birth) were significantly associated with the hormone levels. Testosterone was higher for lower birthweight, SBHG and NSBT were higher for pre-terms, and LH was higher for SGA births, which might be the result of a different timing of the postnatal HPT hormone surge in these boys. The finding of different hormone levels after ART is based on only nine boys, but the lower NSBT level corresponds to a recent longitudinal study in a larger sample (Mau Kai *et al.*, 2007). The latter study differentiated effects between ICSI and IVF, a distinction that was not made in our questionnaire. The lower LH and FSH levels in Turkish subjects may be explained by genetic and environmental factors (Pierik *et al.*, 2004).

In conclusion, the lower testosterone and free-androgen levels in a subgroup of cryptorchidism cases indicate a disturbance of testicular function that is already overt early after birth. Our results provide support for the hypothesis that inhibin B is involved in the regulation of FSH already in infancy. The abnormal hormone levels in boys with suboptimal intra-uterine growth should be follow-up by further study on the implications for future fertility (Barker & Clark, 1997). The finding of lower LH and FSH levels among children from Turkish descent is interesting given their higher risk of cryptorchidism and hypospadias (Pierik *et al.*, 2004). If the lower postnatal LH and FSH can be related to lower levels during fetal development, it may provide a clue to the higher prevalence of both abnormalities in this population. Our findings are based on a single hormone analysis in early postnatal life, and the consequences for later life are still uncertain.

Acknowledgements

The Endocrine Modulators Study Group of the European Chemical Industry Council (EMSG-CEFIC) is acknowledged for financial support. The sponsors of the study had no role in study design, data collection, data interpretation, or reporting.

References

Anderson, R. A. & Sharpe, R. M. (2000) Regulation of inhibin production in the human male and its clinical applications. *International Journal of Andrology* 23, 136–144.

Andersson, A. M. (2000) Inhibin B in the assessment of seminiferous tubular function. *Baillieres Best Practice and Research Clinical Endocrinology and Metabolism* 14, 389–397.

Andersson, A. & Skakkebaek, N. E. (2001) Serum inhibin B levels during male childhood and puberty. *Molecular and Cellular Endocrinology* 180, 103–107.

Andersson, A. M., Juul, A., Petersen, J. H., Muller, J., Groome, N. P. & Skakkebæk, N. E. (1997) Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *Journal of Clinical Endocrinology and Metabolism* 82, 3976–3981.

Andersson, A. M., Toppari, J., Haavisto, A. M., Petersen, J. H., Simell, T., Simell, O. & Skakkebæk, N. E. (1998) Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *Journal of Clinical Endocrinology and Metabolism* 83, 675–681.

Barker, D. J. & Clark, P. M. (1997) Fetal undernutrition and disease in later life. *Reviews of Reproduction* 2, 105–112.

Barthold, J. S., Manson, J., Regan, V., Si, X., Hassink, S. G., Coughlin, M. T. & Lee, P. A. (2004) Reproductive hormone levels in infants with cryptorchidism during postnatal activation of the pituitary-testicular axis. *Journal of Urology* 172, 1736–1741.

Bergada, I., Milani, C., Bedecarras, P., Andreone, L., Ropelato, M. G., Gottlieb, S., Bergada, C., Campo, S. & Rey, R. A. (2006) Time course of the serum gonadotropin surge, inhibins, and anti-Mullerian hormone in normal newborn males during the first month of life. *Journal of Clinical Endocrinology and Metabolism* 91, 4092–4098.

Chada, M., Prusa, R., Bronsky, J., Kotaska, K., Sidlova, K., Pechova, M. & Lisa, L. (2003) Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone during childhood and puberty in males: changes in serum concentrations in relation to age and stage of puberty. *Physiological Research* 52, 45–51.

Crofton, P. M., Illingworth, P. J., Groome, N. P., Stirling, H. F., Swanston, I., Gow, S., Wu, F. C., McNeilly, A. & Kelnar, C. J. (1997) Changes in dimeric inhibin A and B during normal early puberty in boys and girls. *Clinical Endocrinology* 46, 109–114.

Crofton, P. M., Evans, A. E., Groome, N. P., Taylor, M. R., Holland, C. V. & Kelnar, C. J. (2002) Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. *Clinical Endocrinology* 56, 215–221.

Fenichel, P., Rey, R., Poggioli, S., Donzeau, M., Chevallier, D. & Pointis, G. (1999) Anti-Mullerian hormone as a seminal marker for spermatogenesis in non-obstructive azoospermia. *Human Reproduction* 14, 2020–2024.

Gendrel, D., Roger, M. & Job, J. C. (1980) Plasma gonadotropin and testosterone values in infants with cryptorchidism. *Journal of Pediatrics* 97, 217–220.

de Gouveia Brazao, C. A., Pierik, F. H., Erenpreiss, Y., De Jong, F. H., Dohle, G. R. & Weber, R. F. (2003) The effect of cryptorchidism on inhibin B in a subfertile population. *Clinical Endocrinology* 59, 136–141.

Harrell, F. E., Jr. Lee, K. L. & Pollock, B. G. (1988) Regression models in clinical studies: determining relationships between predictors and response. *Journal of the National Cancer Institute* 80, 1198–1202.

John Radcliffe Hospital Cryptorchidism Study Group. (1992) Cryptorchidism: a prospective study of 7500 consecutive male births, 1984–8. John Radcliffe Hospital Cryptorchidism Study Group. *Archives of Disease in Childhood* 67, 892–899.

Lee, P. A. & Coughlin, M. T. (2001) Fertility after bilateral cryptorchidism. Evaluation by paternity, hormone, and semen data. *Hormone Research* 55, 28–32.

Mau Kai, C., Main, K. M., Andersen, A. N., Loft, A., Skakkebaek, N. E. & Juul, A. (2007) Reduced serum testosterone levels in infant boys conceived by intracytoplasmic sperm injection. *Journal of Clinical Endocrinology and Metabolism* 92, 2598–2603.

Pierik, F. H., Vreeburg, J. T. M., Stijnen, T., De Jong, F. H. & Weber, R. F. A. (1998) Serum inhibin B as a marker of spermatogenesis. *Journal of Clinical Endocrinology and Metabolism* 83, 3110–3114.

Pierik, F. H., Burdorf, A., Nijman, J. M., de Muinck Keizer-Schrama, S. M., Juttmann, R. E. & Weber, R. F. (2002) A high hypospadias rate in The Netherlands. *Human Reproduction* 17, 1112–1115.

Pierik, F. H., Burdorf, A., de Jong, F. H. & Weber, R. F. (2003) Inhibin B: a novel marker of spermatogenesis. *Annals of Medicine* 35, 12–20.

Pierik, F. H., Burdorf, A., Deddens, J. A., Juttmann, R. E. & Weber, R. (2004) Maternal and paternal risk factors for cryptorchidism and hypospadias: a case-control study in newborn boys. *Environmental Health Perspectives* 112, 1570–1576.

Pierik, F. H., Burdorf, A., Muinck Keizer-Schrama, S. M. P. F., Wolffenbuttel, K. P., Nijman, J. M., Juttmann, R. E. & Weber, R. F. A. (2005) The cryptorchidism prevalence among infants in the general population of Rotterdam, the Netherlands. *International Journal of Andrology* 28, 248–252.

Raivio, T. & Dunkel, L. (1999) Inverse relationship between serum inhibin B and FSH levels in prepubertal boys with cryptorchidism. *Pediatric Research* 46, 496–500.

Raivio, T. & Dunkel, L. (2002) Inhibins in childhood and puberty. *Best Practice and Research. Clinical Endocrinology and Metabolism* 16, 43–52.

Raivio, T., Toppari, J., Kaleva, M., Virtanen, H., Haavisto, A. M., Dunkel, L. & Janne, O. A. (2003) Serum androgen bioactivity in cryptorchid and noncryptorchid boys during the postnatal reproductive hormone surge. *Journal of Clinical Endocrinology and Metabolism* 88, 2597–2599.

Rey, R. (2000) Assessment of seminiferous tubule function (anti-mullerian hormone). *Baillieres Best Practice and Research. Clinical Endocrinology and Metabolism* 14, 399–408.

de Ronde, W., van der Schouw, Y. T., Pierik, F. H., Pols, H. A., Muller, M., Grobbee, D. E., Gooren, L. J., Weber, R. F. & de Jong, F. H. (2005) Serum levels of sex hormone-binding globulin (SHBG) are not associated with lower levels of non-SHBG-bound testosterone in male newborns and healthy adult men. *Clinical Endocrinology* 62, 498–503.

Rosner, W. (1990) The functions of corticosteroid-binding globulin and sex hormone-binding globulin: recent advances. *Endocrine Reviews* 11, 80–91.

Sharpe, R. M., McKinnell, C., Kivlin, C. & Fisher, J. S. (2003) Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction* 125, 769–784.

Skakkebæk, N. E., Rajpert-De Meyts, E. & Main, K. M. (2001) Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Human Reproduction* 16, 972–978.

Sodergard, R., Backstrom, T., Shanbhag, V. & Carstensen, H. (1982) Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *Journal of Steroid Biochemistry* 16, 801–810.

Suomi, A. M., Main, K. M., Kaleva, M., Schmidt, I. M., Chellakooty, M., Virtanen, H. E. *et al.* (2006) Hormonal changes in 3-month-old cryptorchid boys. *Journal of Clinical Endocrinology and Metabolism* 91, 953–958.

Swerdlow, A. J., Higgins, C. D. & Pike, M. C. (1997) Risk of testicular cancer in cohort of boys with cryptorchidism. *BMJ* 314, 1507–1511.

Tomlinson, C., Macintyre, H., Dorrian, C. A., Ahmed, S. F. & Wallace, A. M. (2004) Testosterone measurements in early infancy. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 89, F558–F559.

Toppari, J., Kaleva, M. & Virtanen, H. E. (2001) Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. *Human Reproduction Update* 7, 282–286.

Toppari, J., Kaleva, M., Virtanen, H. E., Main, K. M. & Skakkebaek, N. E. (2007) Luteinizing hormone in testicular descent. *Molecular and Cellular Endocrinology* 269, 34–37.

Usher, R. & McLean, F. (1969) Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *Journal of Pediatrics* 74, 901–910.

