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ORIGINAL ARTICLE

From mini-puberty to pre-puberty: early impairment of the hypothalamus–pituitary–gonadal axis with normal testicular function in children with non-mosaic Klinefelter syndrome

M. Spaziani[1] · S. Granato[1] · N. Liberati[2] · F. M. Rossi[1] · N. Tahani[1] · C. Pozza[1] · D. Gianfrilli[1] · G. Papi[1] · A. Anzuini[1] · A. Lenzi[1] · L. Tarani[2] · A. F. Radicioni[1]

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Abstract

Purpose Klinefelter syndrome (KS) is a genetic disorder caused by the presence of an extra X chromosome in males. The aim of this study was to evaluate the hypothalamic–pituitary–gonadal (HPG) axis and the clinical profile of KS boys from mini-puberty to early childhood.

Patients and methods In this retrospective, cross-sectional, population study, 145 KS boys and 97 controls aged 0–11.9 years were recruited. Serum FSH, LH, testosterone (T), Inhibin B (INHB), sex hormone binding globulin (SHBG) and anti-Müllerian hormone (AMH) were determined. Auxological parameters were assessed. To better represent the hormonal and clinical changes that appear in childhood, the entire population was divided into 3 groups: ≤ 6 months (group 1; mini-puberty); > 6 months and ≤ 8 years (group 2; early childhood); > 8 and ≤ 12 years (group 3; mid childhood).

Results During mini-puberty (group 1), FSH and LH were significantly higher in KS infants than controls ($p < 0.05$), as were INHB and T (respectively $p < 0.0001$ and $p < 0.005$). INHB was also significantly higher in KS than controls in group 2 ($p < 0.05$). AMH appeared higher in KS than in controls in all groups, but the difference was only statistically significant in group 2 ($p < 0.05$). No significant differences were found in height, weight, testicular volume, and penile length.

Conclusions No hormonal signs of tubular or interstitial damage were found in KS infants. The presence of higher levels of gonadotropins, INHB and testosterone during mini-puberty and pre-puberty may be interpreted as an alteration of the HPG axis in KS infants.

Keywords Klinefelter syndrome · Leydig cells · Sertoli cells · Mini-puberty · Infancy · Hypothalamic · Pituitary · Gonadal axis (HPG)

Introduction

Klinefelter syndrome (KS), first described by Harry Klinefelter in 1942 [1], is the most common sex-chromosomal disorder in males, with a prevalence of 1:660 in the general population [2–4]. It is caused by the presence of an extra X chromosome (80% karyotype 47, XXY; 20% 46, XY/47, XXY mosaicism or structural X chromosome abnormalities). It is also one of the most frequent causes of infertility, affecting 11% of azoospermic and 3.1% of all infertile men [5].

The prevalence of diagnosed KS has risen in recent decades [6], but its true prevalence is still thought to be underestimated, probably due to the extreme variability of its clinical presentation and a lack of awareness among general practitioners [7–10]. Abramsky and Chapple showed that up to 64% of KS patients are never diagnosed, with 10% diagnosed prenatally and only 26% in prepuberty or adulthood [7].

47, XXY males may present with a variety of subtle, age-related clinical signs. Hypospadias, small phallus, cryptorchidism, developmental delay, behavioral problems, incomplete pubertal development with eunuchoid body habitus,

✉ M. Spaziani
matteo.spaziani@uniroma1.it

[1] Department of Experimental Medicine, Section of Medical Pathophysiology, Food Science and Endocrinology, Sapienza University of Rome, Level-1, Room 33, Policlinico Umberto I, 00161 Rome, Italy

[2] Department of Pediatrics, Sapienza University of Rome, 00161 Rome, Italy

gynecomastia, and small testes are its most frequent features in infancy and childhood [11]. Adults are often evaluated for infertility and sexual disorders, but metabolic syndrome, osteoporosis, thyroid dysfunctions, humoral immunoreactivity and the presence of specific personality traits and personality disorders are also described [12–17]. As regards fertility aspects, it has been recently stated that Y chromosome microdeletions do not represent a further negative genetic factor in KS [18].

The function of the hypothalamus–pituitary–gonadal (HPG) axis in KS subjects has already been investigated [19–21], especially in relation to mini-puberty and puberty. Mini-puberty has been investigated extensively, as it is an important temporal window lasting from the first to about the sixth-to-ninth month of life, in which the first significant HPG axis activation takes place [22, 23]. There is literature evidence of prenatal tubular damage [24–27], but these results were not confirmed in childhood biopsies of boys with non-mosaic KS. In 2011, Aksglaede reported that testicular damage begins at the age of 4–9 years with a gradual degeneration of germ cells and reaches its peak from mid-puberty to adulthood, by which time the testes have undergone extensive seminiferous tubule hyalinization and Leydig and Sertoli cell hyperplasia [21]. Literature studies agree that mini-puberty in KS boys is similar to that in healthy boys. However, there is no consensus on the hormonal and clinical profile of young KS patients, nor on when testicular damage occurs.

The aim of this study was therefore to accurately establish the testicular function [28] and the main auxological features over a longer time period, from mini-puberty to the onset of puberty, in order to find any auxological and/or hormonal changes that might be used as an early indicator of KS. This broader clinical and hormonal follow-up might also clarify the specific timing of the onset of puberty, given that while most KS patients present a normal puberty, in some it is delayed and in even more it is precocious.

Patients and methods

Subjects

This was a retrospective, cross-sectional, population study involving 145 pediatric patients (age 0–12 years [mean ± SD: 4.70 ± 3.23]) with a prenatal diagnosis of 47,XXY karyotype, who were followed in the endocrine outpatient clinic (Department of Experimental Medicine) and in the Department of Pediatrics of the Umberto I Polyclinic. The karyotype was confirmed before recruitment, through analysis of postnatal peripheral blood lymphocytes. Karyotypes were established on 40 metaphases from each patient. Three patients (2%) showed cryptorchidism at birth.

The control group consisted of 97 healthy male infants and children (age 0–12 years [mean 5.70 ± 3.46]), recruited from our pediatric and endocrine clinic, where they were under routine growth evaluation. None of them had cryptorchidism, aneuploidies or structural chromosomal abnormalities, nor any endocrine or systemic disorders. For comparison of clinical and hormonal aspects prior to puberty, the two populations were divided into 3 age groups (Table 1): group 1 (age ≤ 6 months: 20 controls aged 0.24–5.64 months [mean 4.10 ± 2.30] and 20 KS patients aged 1.56–5.88 months [mean 3.70 ± 1.20]); group 2 (age > 6 months and ≤ 8 years: 54 controls aged 0.58–7.94 years [mean 4.56 ± 2.28] and 99 KS patients aged 0.51–7.97 years [mean 4.10 ± 2.00]); and group 3 (age > 8 and ≤ 12 years: 23 controls aged 8.10–11.90 years [mean 10.00 ± 1.16] and 26 KS patients aged 8.16–11.89 years [mean 9.86 ± 1.19]). These groups were chosen to best characterize the hormonal and clinical changes that appear in childhood, with group 1 representing infancy (from birth to the end of mini-puberty) and groups 2 and 3 respectively representing early and mid-childhood, with group 3 being considered as the classic pre-pubertal period [23, 29].

Hormone analysis

Baseline blood samples were obtained from all subjects by antecubital venous puncture in the early morning (7.30–9.00 a.m.) after an overnight fast, for determination of serum concentrations of FSH, LH, testosterone (T), inhibin B (INHB), sex hormone binding globulin (SHBG) and anti-Müllerian hormone (AMH). Samples were centrifuged after 30′ and the serum immediately frozen at -20 °C. All tests were performed in duplicate in the laboratory of the Department of Experimental Medicine (Section of Medical Pathophysiology), Sapienza University of Rome. Serum FSH, LH, T and SHBG were measured by chemiluminescent microparticle immunoassay (CMIA, Architect System) (Abbott Laboratories, IL, USA), with limits of detection of 0.05 IU/L, 0.07 IU/L, 0.28 nmol/L, 36.7 pmol/L and 0.1 nmol/L respectively. The intra- and inter-assay coefficients of variation were 3.1 and 4.7% at 3.2 IU/L (FSH), 3.6 and 5.1% at 3.3 IU/L (LH), 2.1 and 3.6% at 10.08 nmol/L (T) and 3.8 and 5.3% at 68.1 nmol/L (SHBG). The normal ranges for prepubertal subjects (i.e. all subjects included in the study) were < 0.05–2.00 IU/L (FSH), < 0.07–1.80 IU/L (LH), < 0.28–2.2 nmol/L (T) and 68.5–228.7 nmol/L (SHBG) [30–32]. Serum INHB was measured using an enzymatically amplified two-site two-step sandwich-type immunoassay (ELISA) (Beckman Coulter, Inc. Brea CA, USA). The limit of detection was 7.0 pg/mL and the intra- and inter-assay coefficients of variation were 3.3% and 7.2% at 122 pg/mL. The normal range for prepubertal subjects was 54.5–250.0 pg/mL [33, 34]. Serum AMH concentration

Table 1 Clinical characteristics of the healthy controls and the Klinefelter patients

Group 1			
	Controls	KS	*p* value
N	20	20	
Age (months)	4.1 ± 2.3	3.7 ± 1.2	0.12
Height (cm)	61 ± 2.8	63 ± 1.5	0.11
Weight (kg)	6.0 ± 0.74	6.5 ± 0.33	0.08
Testicular volume (mL)	1.0 ± 0.10	1.2 ± 0.11	0.23
Penile length (cm)	2.4 ± 0.13	2.4 ± 0.13	0.90
Group 2			
	Controls	KS	*p* value
N	54	99	
Age (years)	4.6 ± 2.3	4.1 ± 2.0	0.16
Height (cm)	105 ± 2.5	103 ± 1.6	0.71
Weight (kg)	18 ± 0.94	18 ± 0.61	0.64
Testicular volume (mL)	1.6 ± 0.08	1.4 ± 0.04	0.09
Penile length (cm)	3.4 ± 0.11	3.6 ± 0.07	0.42
Group 3			
	Controls	KS	*p* value
N	23	26	
Age (years)	10 ± 1.2	9.9 ± 1.2	0.75
Height (cm)	134 ± 2.8	140 ± 2.2	0.06
Weight (kg)	37 ± 4.5	33 ± 1.4	0.07
Testicular volume (mL)	2.8 ± 0.53	2.9 ± 0.17	0.46
Penile length (cm)	5.1 ± 0.67	4.4 ± 0.16	0.83

Data are reported as mean ± SD.

Controls healthy subjects, *KS* Klinefelter patients

was measured using a Gen II enzyme linked immunosorbent assay (ELISA) (Beckman Coulter, Inc. Brea CA, USA) with a limit of detection of 0.57 pmol/L; the intra- and inter-assay coefficients of variation were 5.6% and 7.5% at 94 pmol/L. The normal range for prepubertal subjects was 74–168 pmol/L.

To facilitate data processing, hormone concentrations below the limits of detection were set as follows: 0.03 IU/L for FSH, 0.04 IU/L for LH, 0.20 nmol/L for T and 4 pg/mL for INHB.

The FSH/INHB ratio and the LH/T ratio were evaluated as markers of, respectively, Sertoli and Leydig cell function [35, 36].

Clinical evaluation

Anthropometric evaluation was performed in infants and boys using a stadiometer to measure height. Testicular volume was assessed with the Prader orchidometer. Only KS patients underwent testicular ultrasound, therefore in order to adequately compare the two groups (KS and controls), we used the Prader orchidometer to assess testicular volumes. When the two testes were of different sizes, the mean result was reported. Penile length was measured from the pubic ramus to the tip of the glans.

Ethics

All subjects were followed under a Regional Centre of Rare Diseases' protocol approved as an integrated care pathway by both Policlinico Umberto I and the Lazio Region. Written informed consent was obtained from the participants' parents.

Statistical analysis

The statistical analysis was performed using Prism for windows version 8 (GraphPad software, Inc.). Data in box plots are reported as median, minimum and maximum values (whisker). Unpaired two-tailed T tests or, when appropriate,

non-parametric tests (Mann Whitney test) were used for comparisons of the data after testing for normal distribution. Statistical significance was set at 95% confidence interval ($p < 0.05$). The Pearson correlation test was used to verify correlations between two hormones.

Results

All results are reported as mean ± SD unless otherwise indicated. The statistical analysis demonstrated that there were no differences in age, height, weight, testicular volume or penile length between KS and controls for each age-correlated group (Table 1). The KS patients also underwent testicular ultrasound; none of them showed any testicular abnormalities or testicular focal lesions. One patient in group 2 had testicular microlithiasis. The results of the hormonal evaluation in controls and KS subjects are reported in Table 2.

Sertoli cells function

Plasma levels of FSH were higher in KS patients than in the controls in group 1 (1.2 ± 0.50 vs 0.68 ± 0.30; $p = 0.0002$). No statistically significant differences were observed in the other two groups, although KS subjects showed higher FSH levels than controls in both group 2 and group 3 (Fig. 1a, b). INHB plasma levels were significantly higher in KS patients than in controls in group 1 (229 ± 82 vs 105 ± 45; p < 0.0001) and in group 2 (91 ± 43 vs 71 ± 31; $p < 0.01$) (Fig. 1c), and were higher than the 90th percentile of control population in 87.5% of group 1 subjects, in 24% of group 2 subjects and in 12% of group 3 subjects. Furthermore, in group 1, there was a strong

Table 2 Hormone values of the healthy controls and the Klinefelter patients

Group 1			
	Controls	KS	*p* value
N	20	20	
FSH (mIU/mL)	0.68 ± 0.30	1.20 ± 0.50	*= 0.0002*
LH (mIU/mL)	0.84 ± 0.75	1.40 ± 0.94	*< 0.05*
T (nmol/L)	1.10 ± 0.82	3.30 ± 1.80	*< 0.05*
SHBG (nmol/L)	131 ± 70	135 ± 48	0.69
INHB (pg/mL)	105 ± 45	229 ± 82	*< 0.0001*
AMH (pmol/L)	120.5 ± 44.2	146.5 ± 54.1	0.28
Group 2			
	Controls	KS	*p* value
N	54	99	
FSH (mIU/mL)	0.39 ± 0.25	0.60 ± 0.59	0.18
LH (mIU/mL)	0.06 ± 0.06	0.07 ± 0.06	0.21
T (nmol/L)	0.27 ± 0.14	0.24 ± 0.18	0.13
SHBG (nmol/L)	112 ± 48	135 ± 40	*< 0.005*
INHB (pg/mL)	71 ± 31	91 ± 43	*< 0.01*
AMH (pmol/L)	115 ± 8.7	150 ± 8.6	*< 0.05*
Group 3			
	Controls	KS	*p* value
N	23	26	
FSH (mIU/mL)	1.13 ± 0.69	1.19 ± 0.81	0.14
LH (mIU/mL)	0.24 ± 0.31	1.20 ± 2.31	0.26
T (nmol/L)	0.67 ± 0.39	1.97 ± 2.88	0.09
SHBG (nmol/L)	84 ± 57	95 ± 46	0.41
INHB (pg/mL)	78 ± 32	98 ± 46	0.15
AMH (pmol/L)	96.9 ± 47.3	103.9 ± 49.1	0.40

Data are reported as mean ± SD; Italic values indicate signifcant differences ($p < 0.05$)

Controls healthy subjects, *KS* Klinefelter patients

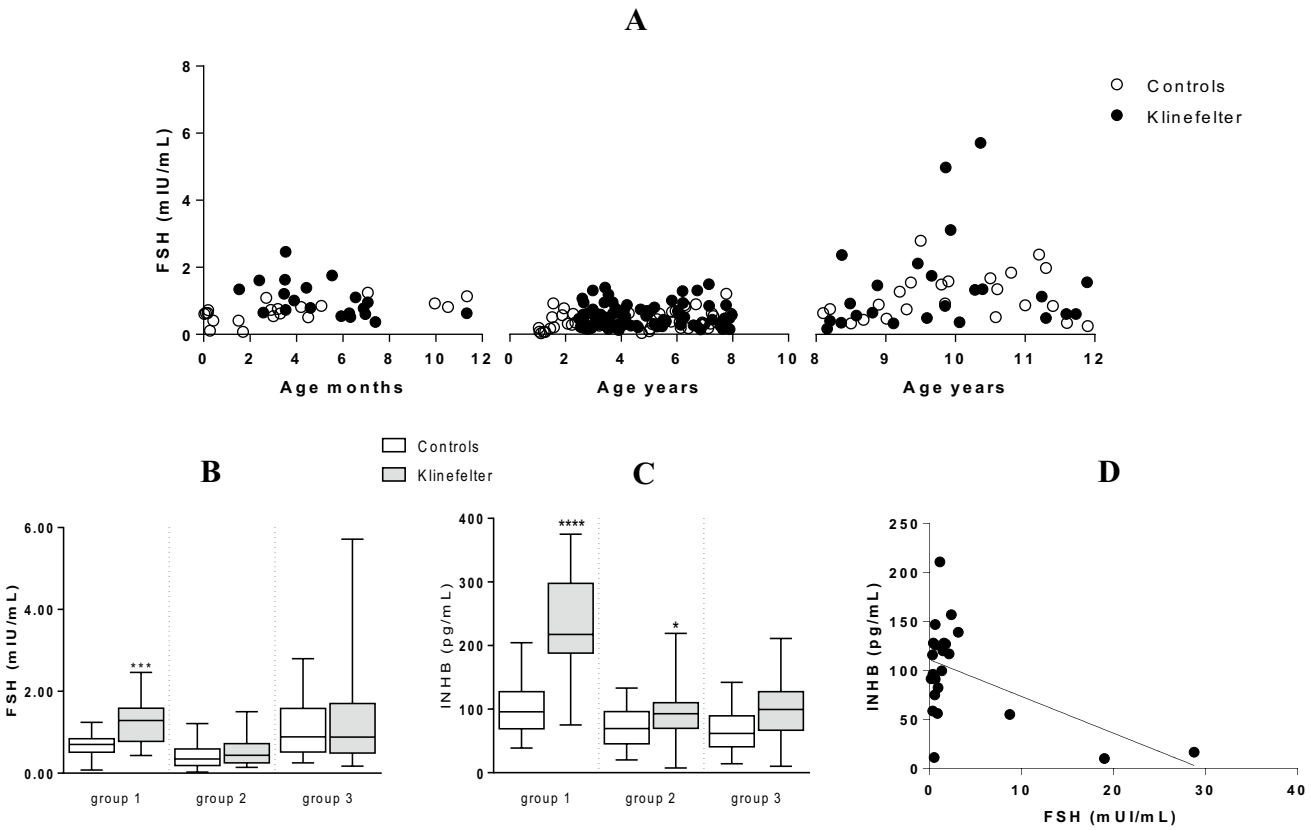

Fig. 1 **a** FSH levels according to chronological age in controls (white circles) and Klinefelter patients (black circles). **b**, **c** Box plots with median, minimum and maximum values (whiskers) of serum FSH (**b**) and INHB (**c**) concentrations in controls (white boxes) and KS patients (gray boxes). Comparison *p* values are also reported: ***$p=0.0002$; ****$p<0.0001$; *$p<0.01$. **d** Correlation between serum levels of FSH and INHB in KS patients in group 3. Regression lines are shown, although Pearson correlation coefficients were computed. ($r^2=0.28$, $p=0.007$)

association between KS and INHB values higher than the 75th percentile of the control population (OR 40–95% CI 3.6–450; $p<0.001$). There was no difference in the FSH/INHB ratio between KS and controls in any group. As expected, there was a positive correlation between FSH and INHB in group 1 controls ($r^2=0.55$, $p=0.01$), while no correlation was found in KS patients: in contrast, there was a significant negative correlation between FSH and INHB in KS subjects in group 3 ($r^2=0.28$, $p=0.007$) (Fig. 1d).

AMH concentrations in KS patients were always higher than in the controls in all the three groups, but this difference was only significant in group 2 (150 ± 8.6 vs 115 ± 8.7; $p<0.05$) (Fig. 2a). In the KS population, there was a statistically significant negative correlation between FSH and AMH in groups 1 and 3 (respectively $r^2=0.41$, $p<0.003$ and $r^2=0.34$, $p<0.002$) (Fig. 2b, c). There was a statistically significant positive correlation between INHB and AMH in both controls and KS patients in group 2 (respectively $r^2=0.26$, $p<0.002$ and $r^2=0.31$, $p<0.0001$) and in KS subjects alone in group 3 ($r^2=0.24$, $p<0.05$) (Fig. 3a, b, c).

Leydig cells function

There was a statistically significant higher LH concentration in KS subjects than controls in group 1 (1.4 ± 0.94 vs 0.84 ± 0.75; $p<0.05$), while the difference was not statistically significant in group 3. No differences were found in group 2, where LH was very low in both KS patients and controls (Fig. 4a, b). T concentrations followed a similar trend: T was significantly higher in KS patients than controls in group 1 alone (3.3 ± 1.8 vs 1.1 ± 0.82; $p<0.05$), while the difference was not statistically significant in group 3, although KS subjects did have a higher average T concentration (Fig. 4c). T values in KS subjects were higher than the 90th percentile of the control population in 43% of group 1 subjects, in 2% of group 2 subjects and in 27% of group 3 subjects. There was a strong association between KS and T values higher than the 75th percentile of control population in group 1 (OR 21–CI 95% 2.4–184, $p<0.005$), while no associations were found in the other groups.

No difference was found in the LH/T ratio between KS and controls in any group. There was a significant positive

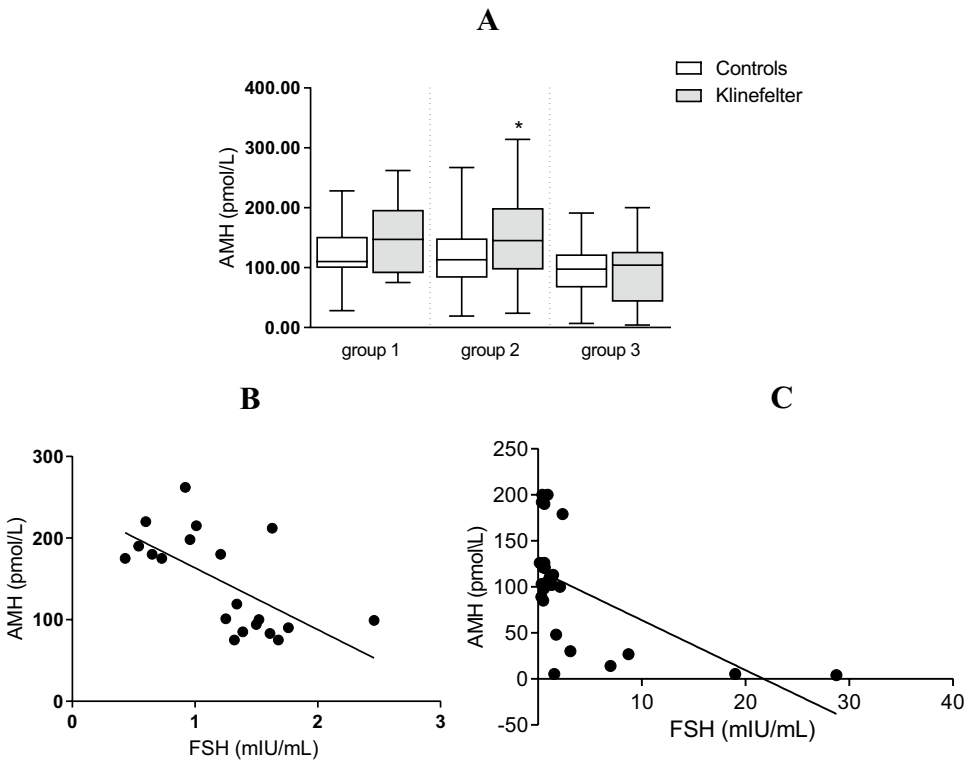

Fig. 2 **a** Box plots with median, minimum and maximum values (whiskers) of serum AMH concentrations in controls (white boxes) and KS patients (gray boxes). Comparison p values are also reported: *$p < 0.05$. **b**, **c** Correlation between serum levels of FSH and AMH in KS patients in groups 1 and 3 respectively. Regression lines are shown, although Pearson correlation coefficients were computed. (respectively $r^2 = 0.41$, $p < 0.003$ and $r^2 = 0.34$, $p < 0.002$)

correlation between LH and T in controls and KS subjects in group 1 (respectively $r^2 = 0.32$, $p < 0.001$ and $r^2 = 0.51$, $p = 0.004$) (Fig. 5a, b), and in group 3 (respectively $r^2 = 0.28$, $p < 0.001$ and $r^2 = 0.88$, $p < 0.0001$) (Fig. 5c, d). SHBG concentrations were significantly higher in KS patients than controls in group 2 (135 ± 40 vs 112 ± 48; $p < 0.005$).

Discussion

To our knowledge, this is the first study that specifically investigates the hormonal changes during the three phases of childhood considered individually. We studied circulating levels of hormones in relation to chronological age in non-mosaic 47, XXY patients aged 0–12 years. The hormonal changes that take place in KS patients at the onset of puberty have been broadly studied and discussed in the literature, but there is less information on what happens during the two phases of pre-puberty (early and mid-childhood).

Our data show that there are subtle differences even between mid- and late childhood in both KS patients and healthy boys. This demonstrates that the age subdivisions used herein enable a more refined, detailed analysis of the prepubertal period: the existing literature data, in fact, describe the hormonal changes in pediatric KS patients over a time period that may be too broad to identify more subtle hormone changes.

Sertoli cells function

The expected physiological surge in FSH and INHB during infancy—the well-known mini-puberty—was observed in both KS subjects and healthy boys. However, their levels were higher in KS infants than in controls, and this pattern was also confirmed in older boys (aged 6 months to 12 years), although the only statistically significant difference in these boys was for INHB in group 2 (see Fig. 1c).

These results do not seem to demonstrate the classic Sertoli cell resistance at a very early age described in several studies [37–39]. Cabrol found greater FSH levels in some KS patients, associated with sub-normal INHB levels (19% of their aneuploid population had INHB below the 5th percentile of healthy infants), which could explain the reduced Sertoli cell sensitivity, a forerunner of the classic pubertal gonad damage [37].

Aksglaede and coworkers [38] studied 10 KS patients aged 1.8–3.8 months, comparing them to 613 age-matched healthy subjects. They found an increased FSH/INHB ratio in the KS patients, a sign of a subtle Sertoli cell dysfunction.

Lahlou et al. [39] also found a certain degree of Sertoli cell dysfunction, with 13 of 77 KS patients enrolled in their study (age < 2 years) presenting INHB levels below the 5th percentile of controls, despite normal levels of FSH. A previous study by Lahlou [18] found no differences in INHB levels between KS patients and control groups, in agreement with Aksglaede's results [38]. In both papers [18, 38], the

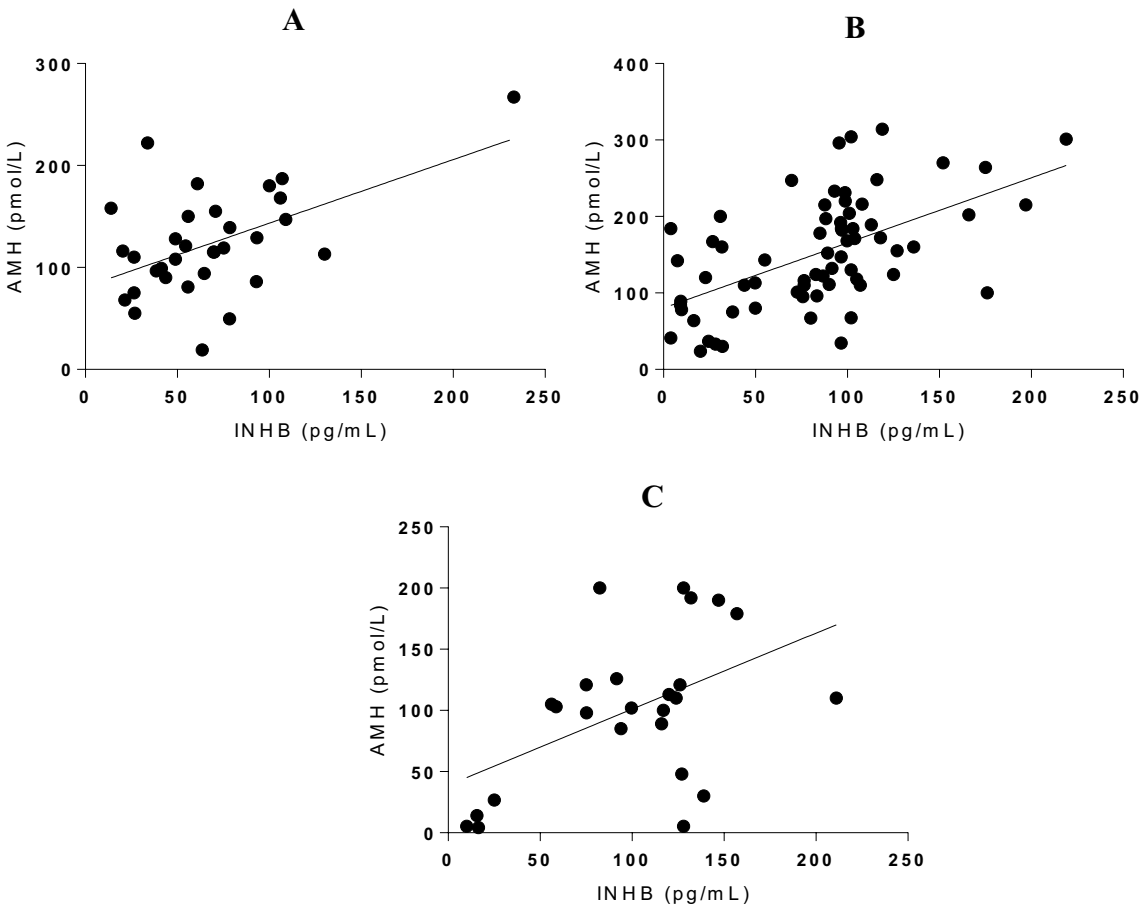

Fig. 3 Correlation between serum levels of INHB and AMH in controls and KS patients in group 2 (**a**, **b**) and in KS patients in group 3 (**c**). Regression lines are shown, although Pearson correlation coefficients were computed. (respectively $r^2=0.26$, $p<0.002$; $r^2=0.31$, $p<0.0001$; $r^2=0.24$, $p<0.05$)

authors demonstrated a positive correlation between FSH and INHB, in contrast with our findings: the only significant correlation we found was a negative one, for KS subjects in group 3 (aged 8 to 12 years). Another important difference was our observation of significantly higher INHB levels in KS patients in groups 1 and 2; in group 1 (mini-puberty), as noted above, we were unable to confirm the previously reported correlation between FSH and INHB in KS subjects, although this correlation was found in the control group. We did find a significant positive correlation between FSH and INHB (as was also seen in the control population) when considering the KS population as a whole, demonstrating that INHB secretion in non-mosaic XXY children is still sensitive to physiological FSH stimulation. Even though we did not find any correlation between FSH and INHB during mini-puberty, these data seem to demonstrate that Sertoli cells are not only still able to respond to increased gonadotropin stimulation in this phase, but their response is even higher than in the controls, as demonstrated by the elevated INHB levels. In fact, in KS subjects higher INHB levels are associated with higher FSH concentrations, particularly during mini-puberty, as if the pituitary pacing was set at a higher level. This suggests the probable impairment of the HPG axis and/or compartmental cross-talking in the gonads. FSH and INHB levels in KS subjects both followed a similar pattern to the controls, tending to drop after their peak in mini-puberty, although they still remained higher than in the controls (Fig. 1).

Studies in the literature report normal or high AMH levels in KS infants, with its secretion increasing progressively from the time of birth, peaking between 1 month and 1 year of age [18, 37]. We found that not only AMH levels in KS patients were within the normal ranges in all age groups, but that in group 2 they were actually significantly higher in KS subjects than in controls (Fig. 2a). There was an almost perfect concordance between INHB and AMH trends in KS patients in all three groups. In group 2 both AMH and INHB were significantly higher in KS subjects than in controls, while in both group 1 (mini-puberty) and group 3 there was a greater difference between KS and controls for INHB

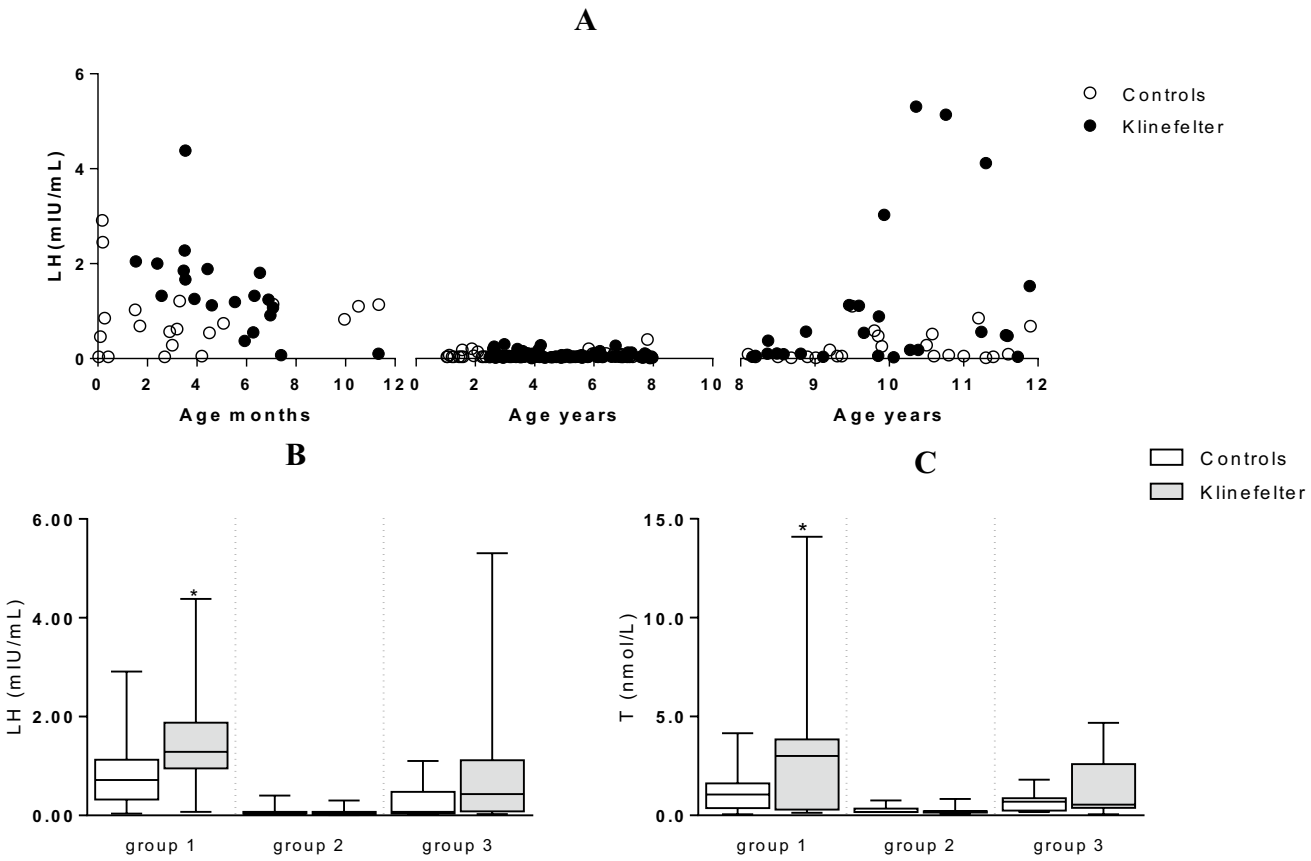

Fig. 4 **a** LH levels according to chronological age in controls (white circles) and Klinefelter patients (black circles). **b**, **c** Box plots with median, minimum and maximum values (whiskers) of serum LH (**b**) and T (**c**) concentrations in controls (white boxes) and KS patients (gray boxes). Comparison *p* value is also reported: $*p < 0.05$

values, but not for AMH. Such observations, which are in agreement with the published data, demonstrate that the rise in testosterone during mini-puberty does not have the same suppressive effect on Sertoli cells as seen in puberty [37, 38, 40]. This strengthens the theory that Sertoli cells do not express androgen receptors in infancy [41, 42].

We also found a significant negative correlation between FSH and AMH in KS subjects in both mini-puberty's group 1 and group 3. Analysis of Fig. 2b, which shows this negative correlation during mini-puberty, suggests that there are two hypothetical groups of subjects: those with low FSH/high AMH, and those with high FSH/low AMH. These two groups could demonstrate the possible presentations of KS infants during mini-puberty: subjects with seemingly more sensitive Sertoli cells secrete high/normal levels of AMH under the stimulus of low FSH concentrations, while subjects with a certain degree of Sertoli cell resistance require higher FSH concentrations to secrete even a reduced AMH level. However, a greater number of subjects is needed to strengthen or weaken this hypothesis.

Lahlou [18] excluded a direct correlation between FSH and AMH, but underlined the proliferative and secretive effects of FSH on Sertoli cells, with a consequent increase in AMH production that persists beyond the classic reduction in FSH levels occurring after mini-puberty. We found a statistically significant positive correlation between INHB and AMH in both controls and KS subjects in group 2 and in KS alone in group 3. Unlike Lahlou [18], we did not find any correlations during mini-puberty. All the published reports seem to exclude the hypothesis that, unlike healthy subjects, AMH secretion in KS subjects is regulated through a partly different mechanism to INHB. It is however evident that AMH secretion is more regular and stable than INHB.

Leydig cells function

Several studies describe the physiological rise in LH in KS infants during mini-puberty and its subsequent drop to undetectable levels until puberty [20, 21, 37]. In particular, Cabrol and co-authors did not find any changes in Leydig cell proliferation and differentiation, as INSL-3 levels were similar to controls and showed a positive correlation with LH and testosterone concentration [37], while Aksglaede described elevated levels of LH in KS patients until puberty

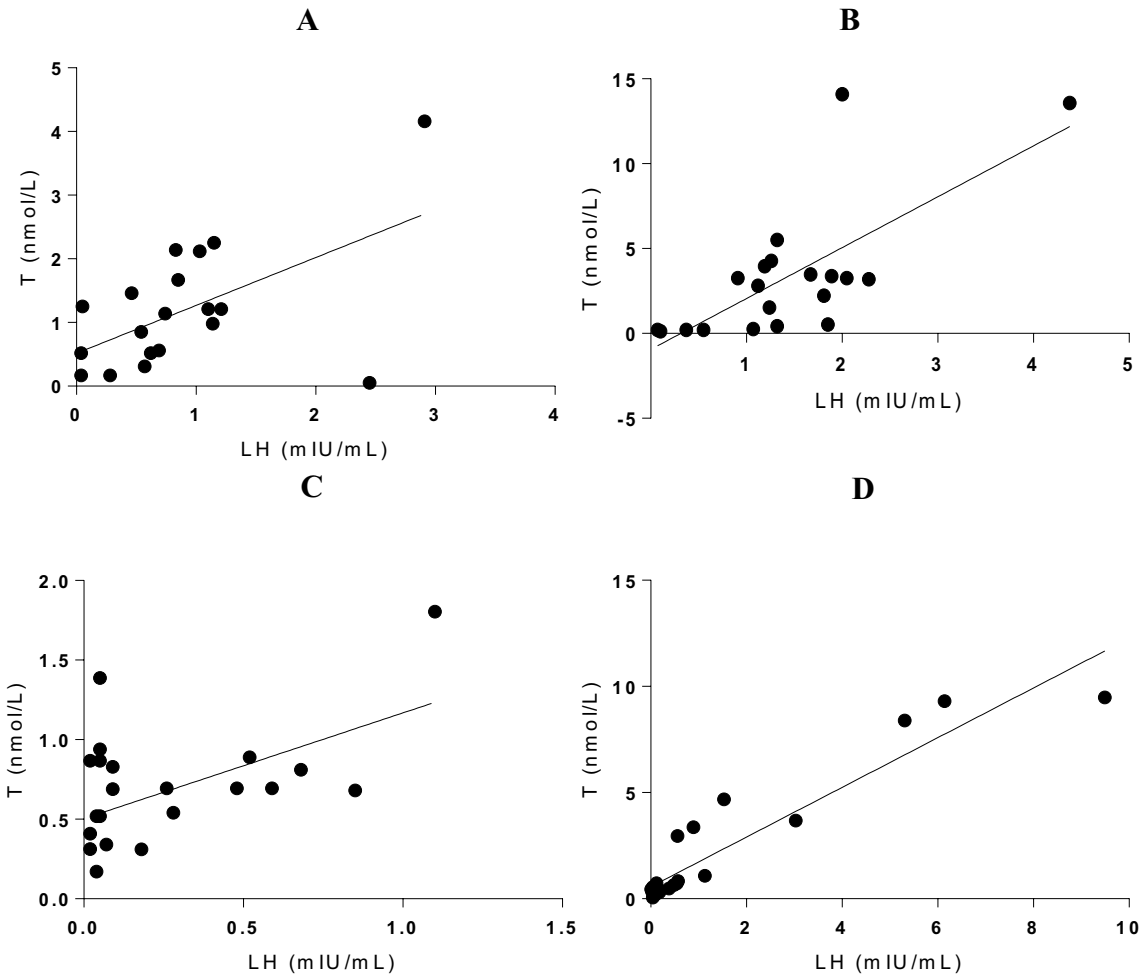

Fig. 5 Correlation between serum levels of LH and T in controls and KS patients in group 1 (**a**, **b**) and group 3 (**c**, **d**). Regression lines are shown, although Pearson correlation coefficients were computed. (respectively: $r^2=0.32$, $p<0.001$; $r^2=0.51$, $p=0.004$; $r^2=0.28$, $p<0.001$; $r^2=0.88$, $p<0.0001$)

[38]. We found LH to be significantly greater in KS patients than in controls in mini-puberty's group 1, with a non-significant difference in group 3 and no difference in group 2. In group 1, LH was above the 75th percentile of controls in 50% of KS patients.

Literature reports of testosterone levels in KS boys are conflicting. In the present study the expected physiological surge of T during mini-puberty was observed, with significantly higher T concentrations in group 1 KS patients. There was also a significant positive correlation between LH and T in both patients and controls in groups 1 and 3. Various authors have reported the expected increase in T during mini-puberty in KS infants, whereas Lahlou and Ross both described an attenuated surge, indicating T deficiency [18, 43]. In contrast to his 2004 paper, Lahlou et al. [39] described a physiological T surge in KS infants during the neonatal period, although it was on average lower than in the controls; the authors concluded that Leydig cells are in any case sensitive to the proliferative effects of LH. Aksglaede described significantly higher LH levels in KS infants than age-matched controls. These were associated with high normal serum T levels, again significantly higher in the KS infants, suggesting an alteration in the pituitary-Leydig cell set point, but not a Leydig cell dysfunction. The authors suggested a Sertoli cell dysfunction represented by a significantly higher FSH/INHB ratio, causing an increase in GnRH which itself induced a rise in both FSH and LH, whose stimulating effect on Leydig cells produces an increase in T concentrations [38].

We did not find any statistically significant difference in T between KS and healthy boys in group 2 and 3, while there was a significant correlation between T and LH in both KS boys and controls, confirming the physiological interstitial response to pituitary stimulation. These LH-T axis data suggest hyperstimulation of the Leydig cells during mini-puberty in KS patients, which could encourage increased

testosterone secretion during early infancy: this is probably caused by the same pathophysiological mechanism already seen and described for the FSH-INHB axis. Another possible explanation may be linked to androgen receptor (AR) CAG polymorphism, caused by microsatellite trinucleotide CAG repeats. It is well known that AR transactivation activity is inversely associated with the number of CAG repeats (normal range 11–31), so in those patients with a higher number of CAG repeats, more gonadotropin stimulation may be necessary to overcome the reduced AR activity. In other words, subtle decreases in AR activity due to longer CAG repeats are counterbalanced by subtle upregulation of the activity of the hypothalamic-pituitary–gonadal axis [44].

With the exception of group 2, there was also no significant difference in SHBG values, which showed considerable scattering.

Clinical observations

Although the comparison of the two populations in terms of auxological data did not show statistically significant differences (Table 1), the KS children in the present study were of greater body weight and length than the controls up to the age of 0.75 years, with the difference being statistically significant (data not shown). KS children in group 3 were also taller than age-matched controls, although this difference was not statistically significant. Bastida and Ross [40, 43] also found KS children to be taller, although the latter described short penile length and low testicular volume, as also reported by Pacenza [45]. As previously mentioned, we did not find statistically significant differences, between the two populations of the present study, for both penile length and testicular volume (Table 1). In contrast, Aksglaede found KS children to be shorter and lighter [46].

Finally, our data showed that an anticipated onset of puberty is possible in Klinefelter syndrome, since many patients in group 3 (aged between 8 and 12 years), the classic pre-pubertal period, had a higher T concentration than the controls, although this difference was not statistically significant. Auxological evaluation of these patients in fact revealed a more advanced Tanner pubertal stage.

Conclusions

No hormonal signs of tubular or interstitial damage was found in KS infants. The presence of higher gonadotropin and testosterone levels during mini-puberty may be interpreted as an altered HPG axis in KS infants, perhaps associated with the reduced AR activity. This study of gonadal function in a population of young boys with Klinefelter syndrome demonstrates the importance of following boys at all ages, from birth until pre-puberty.

The analysis of Sertoli cell function suggested that AMH and INHB are secreted in very different ways. On average, AMH levels were steadily high in all KS boys in all age groups, but the same was not true for INHB, which was higher in group 1 and lower in group 2 and 3.

Higher FSH levels were seen in a large number of KS boys. We exclude the hypothesis that the elevated FSH levels associated with high or normal INHB may be the consequence of partial Sertoli cell resistance. Rather, we believe that it is probably the result of an altered HPG axis and/or compartmental cross-talking in the gonads, with the pituitary pacing set at a higher level. The latter aspect is confirmed by the higher LH values; these interact with Leydig function, resulting in an increased testosterone secretion in some patients.

In conclusion, our study demonstrated that neither Sertoli cells nor Leydig cells present signs of dysfunction at an early age but, on the contrary, display an appropriate response to the increased FSH and LH stimulation by the secretion of INHB, AMH and T respectively. It also confirmed the extreme variability in the clinical features of infantile and pre-pubertal KS subjects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Policlinico Umberto I Ethics Committee (Sapienza University of Rome).

Informed consent Informed consent was obtained from all individual participants included in the study.

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