Inhibin B and Anti-Müllerian Hormone, But Not Testosterone Levels, Are Normal in Infants with Nonmosaic Klinefelter Syndrome

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Klinefelter syndrome is a major cause of infertility in the male. Nevertheless, pregnancies were recently obtained by intracytoplasmic injection of sperm retrieved by surgery or ejaculation, underscoring the need to understand the role of Sertoli and Leydig cell secretions during development. In 18 infants with prenatally diagnosed homogenous 47,XXY karyotype, blood samples were taken from birth to 3 yr of age. Inhibin B (INHB), anti-Müllerian hormone (AMH), testosterone, FSH, and LH levels were compared with those in six adolescents with XXY karyotype and reference values established in 215 control infants. In XXY infants FSH, LH, INHB, and AMH did not differ from controls. Testosterone levels during the first trimester exhibited a physiological increase but were lower than in controls ($P = 0.0001$). Significant correlations were found between testosterone and LH ($P < 0001$), between INHB and FSH ($P = 0.0011$), and between AMH and INHB ($P = 0.025$). In XXY adolescents, AMH and INHB were undetectable. Our findings demonstrate that testosterone secretion is impaired in infants with Klinefelter syndrome. By contrast, INHB and AMH secretions were not altered, which raises the question of the mechanism(s) governing the decline of Sertoli cell function after puberty. (*J Clin Endocrinol Metab* 89: 1864–1868, 2004)

KLINEFELTER SYNDROME IS a major cause of infertility in the male. In 1959 the syndrome was related to a specific chromosome abnormality with affected subjects bearing an extra X chromosome. The 47,XXY karyotype is usually acquired through a nondisjunction during parental gametogenesis, of either paternal (53%) or maternal (44%) origin. In only 3% of the cases, the origin is an error in postzygotic division, which results in mosaicism (review in Ref. 1). The syndrome affects about 1 in 500 boys (2, 3) and is responsible in most cases for complete azoospermia. Until recently the syndrome was recognized only at adolescence in the presence of characteristic clinical symptoms: tall stature, persistent pubertal gynecomastia, small testes, and azoospermia. Mental retardation has also been reported in a number of cases (1, 4). The main biological features, such as elevated gonadotropins and decreased testosterone, have been known for a long time (review in Ref. 1). The lack of inhibin secretion, which was more recently reported (5), emphasized the defect in mature Sertoli cell function in this syndrome.

A renewed interest in testis function in Klinefelter subjects arose from the reports of successful pregnancies obtained in nonmosaic 47,XXY subjects by intracytoplasmic sperm injection after surgical sperm extraction or ejaculation (review in Ref. 6). There are still some controversies regarding the possibility of intratesticular mosaicism in subjects with the nonmosaic 47,XXY blood karyotype. However, these results request further assessment of testis functions from birth to adulthood, with the aim of bringing future assistance to parents in their decision to continue pregnancy, when the chromosomal abnormality was prenatally diagnosed.

The aim of this work was to investigate early in life the secretion of Sertoli and Leydig cell hormones. Owing to prenatal diagnosis of Klinefelter syndrome, we had the opportunity to compare hormonal data in a significant number of 47,XXY infants and to a large cohort of normal infants and to demonstrate that Sertoli cell function appears to be normal in infants with nonmosaic Klinefelter syndrome, in contrast to impaired Leydig cell secretion.

Subjects and Methods

Subjects

Eighteen infants with homogenous 47,XXY karyotype were investigated for 1–3 yr (mean observation time, 610 d). The syndrome has been prenatally diagnosed by amniocentesis in the course of screening for Down syndrome or during the search of other chromosomal disorders. After comprehensive genetic counseling, including information on the high risk of infertility, parents chose to continue the pregnancy. They gave informed consent for a clinical and biological postnatal follow-up. The infants had normal height and weight at birth. Regular clinical and psychological consulting took place during the first 3 yr of life. Their growth rate and mental development for the period of observation was comparable with those of male infants with normal karyotype. Blood was collected at various intervals, one to three times during the first 3 yr of observation. A total of 50 individual samples were collected and distributed among five age groups, first month and 1–3, 4–8, 9–18, and 19–36 months. Their biological data were compared with reference values established in our laboratory from 215 infants investigated for various reasons and found to be free of any endocrine or metabolic disorder. In addition, gonadotropins and testis hormones were measured in six adolescents with nonmosaic 47,XXY karyotype, aged 14–18 yr.

TABLE 1. Evolution of hormonal parameters according to age in 18 infants and in six adolescents with nonmosaic Klinefelter syndrome

Age	1–30 d	1–3 months	4–8 months	9–18 months	19–36 months	14–18 yr
No. of samples	9	10	10	11	10	6
FSH (IU/liter)	2.22 ± 0.24	2.06 ± 0.40	1.76 ± 0.50	0.43 ± 0.11	0.59 ± 0.16	31 ± 2.2
	(1.7–3.5)	(0.6–5.2)	(0.13–3.4)	(0.01–1.1)	(0.02–1.6)	(23–36)
	[0.2–3.5]	[0.2–4.0]	[0.1–3.2]	[0.1–1.5]	[0.01–1.6]	[0.6–4.8]
LH (IU/liter)	4.4 ± 0.73	1.93 ± 0.48	0.58 ± 0.17	0.18 ± 0.03	0.08 ± 0.03	21 ± 1.8
	(2.7–7.6)	(0.12–4.8)	(0.05–1.1)	(0.01–0.35)	(0.02–0.28)	(15–29)
	[0.5–6.5]	[0.5–7.1]	[0.2–3.7]	[0.01–1.3]	[0.01–0.8]	[0.9–2.0]
Testosterone (ng/ml)	0.91 ± 0.18	1.17 ± 0.20	0.33 ± 0.12	0.04 ± 0.01	0.04 ± 0.01	1.59 ± 0.32
	(0.37–1.73)	(0.29–1.90)	(0.06–0.49)	(<0.02–0.09)	(<0.02–0.11)	(1.12–2.51)
	[0.10–2.91]	[0.52–4.79]	[<0.02–1.50]	[<0.02–0.28]	[<0.02–0.15]	[1.21–7.01]
INHB (pg/ml)	179 ± 22	275 ± 30	205 ± 23	110 ± 12	42 ± 7	<6
	(112–289)	(144–454)	(104–289)	(52–173)	(15–90)	(<6–10)
	[75–575]	[125–570]	[70–350]	[30–220]	[17–94]	[100–330]
AMH (ng/ml)	58 ± 4.76	119 ± 8.96	124 ± 5.04	103 ± 12.7	74.2 ± 7.98	<0.35
	(42.6–79.3)	(58.1–150)	(111–153)	(41.9–187)	(37.7–106)	(<0.35)
	[25.2–154]	[36.4–162]	[37.8–168]	[39.9–168]	[33.6–168]	[3.9–78.4]

Values are expressed as mean ± SEM (range). Reference values in controls are within *brackets*. The conversion factors from metric units to SI units are as follows: testosterone, nanomoles per liter, multiply by 3.4674; AMH, nanograms per milliliter to picomoles per liter, multiply by 7.14.

Methods

Blood was collected in vacuum tubes, and the serum was immediately separated from cells and frozen. The specimens were shipped in dry ice and stored at −20 C until assayed.

FSH and LH were measured by a time-resolved fluorometric assay using Delfia reagents (Perkin Elmer Life Sciences, Courtaboeuf, France). Sensitivity for both assays was 0.01 IU/liter. Testosterone was measured by direct RIA using Orion reagents (CisBio International, Gif sur Yvette, France). Sensitivity was 0.01 ng/ml (0.05 nmol/liter). Inhibin B (INHB) was measured by an enzyme immunometric assay using Oxford Bio-Innovation reagents (Argene Biosoft, Varilhes, France), as described previously (7). Sensitivity was 6 pg/ml. Anti-Müllerian hormone (AMH) was measured by an enzyme immunometric assay using Immunotech reagents (Beckman Coulter, Villepinte, France). Sensitivity was 0.35 ng/ml (2.5 pmol/liter). SHBG was measured by means of Delfia reagents (Perkin Elmer Life Sciences). Sensitivity was 0.5 nmol/liter.

Statistics

The effects of age and clinical status (Klinefelter or control) were assessed by ANOVA using the Bonferroni/Dunn test. Correlations between hormone parameters were computed by means of the Spearman's rank test.

Results

Both FSH and LH levels in Klinefelter infants were significantly higher in the period from birth to 3 months than in 4–18 month olds ($P = 0.0012$); they did not differ from controls (Table 1).

Testosterone exhibited a physiological increase during the first months of life, like in normal boys (Fig 1), but at a lower level than in controls from birth to 8 months ($P = 0.0001$). Testosterone levels were significantly correlated with LH ($r = 0.726$, $P < 0.0001$).

SHBG levels, measured in Klinefelter subjects and matched controls aged 1–8 months, did not differ significantly (80–180 *vs.* 70–190 nmol/liter, $P > 0.40$).

INHB levels (Fig. 2) increased significantly from birth to 4 months ($P = 0.04$) and then decreased progressively; they did not differ from controls. They were strongly correlated with FSH levels ($r = 0.607$, $P = 0.0011$) as in controls ($r = 0.58$, $P = 0.0009$). In an XXY boy who was given im testosterone enanthate (100 mg/m^2) on d 40 and on d 55 of age, both FSH
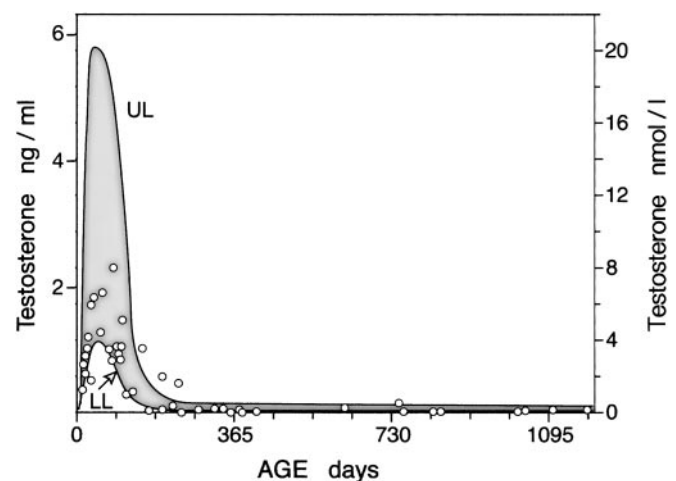

FIG. 1. Testosterone levels according to age in 47,XXY infants. *Hatched area*, Range of reference values in control infants (LL, lower limit; UL, upper limit = smoothed 5th and 95th percentiles). To convert nanograms per milliliter to nanomoles per liter, multiply by 3.4674.

and INHB levels markedly declined and then increased as soon as normal FSH was restored (Fig 3).

AMH levels increased progressively (Fig 4) from birth to 8 months ($P = 0.04$) and then remained stable during the observation delay of 2–3 yr. They did not differ from controls, and they were correlated with neither FSH nor LH levels. There was no reverse relationship between testosterone and AMH levels ($r = 0.02$, $P = 0.88$), similar to control infants ($r = 0.24$, $P = 0.28$). On the other hand, despite different kinetics during the entire observation, AMH levels were significantly correlated with INHB ($r = 0.39$, $P = 0.025$), similar to control infants ($r = 0.60$, $P < 0.001$).

Discussion

We report here for the first time the kinetic evolution of gonadotropin and testis hormone levels in infants with prenatally diagnosed nonmosaic Klinefelter syndrome. Consistent with previous observations in adolescent and adult
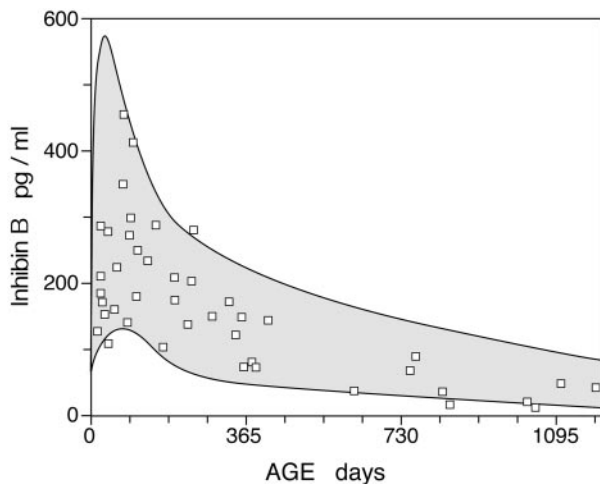
FIG. 2. Inhibin B levels according to age in 47,XXY infants. *Hatched area*, Range of reference values in control infants (same symbols as in Fig. 1).

FIG. 4. AMH levels according to age in 47,XXY infants. *Hatched area*, Range of reference values in control infants (same symbols as in Fig. 1).
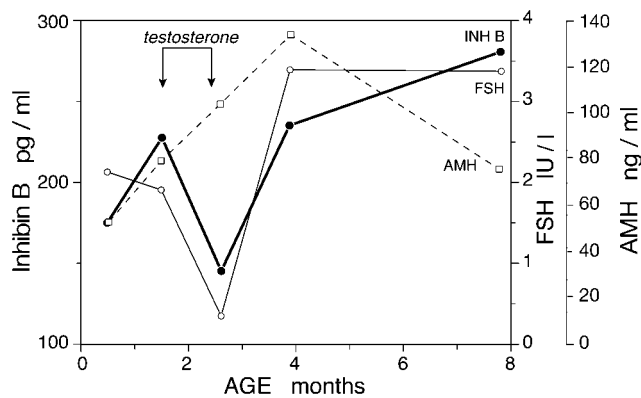

FIG. 3. Changes in INHB, AMH, and FSH levels from birth to 8 months of age in an infant with nonmosaic 47,XXY karyotype, who has been treated with testosterone for 1 month (100 mg testosterone enanthate IM per square meter on d 40 and d 55 of age). INHB levels were markedly sensitive to changes in FSH levels. During testosterone administration, AMH level increased from 79–104 ng/ml despite the marked increase in testosterone level from 1.2–2.3 ng/ml (data not shown). All the observed changes in serum levels were far beyond intraassay variability.

Klinefelter subjects, testosterone levels in the neonatal period were lower than in normal infants. However, the secretory profile exhibited the same kinetics as in controls. By contrast, Sertoli cell hormones, AMH, and INHB exhibited physiological kinetics and amplitude characteristic of the first postnatal trimester, similar to those in controls.

Leydig cell secretion of testosterone is impaired early in infants with Klinefelter syndrome

The impairment of testosterone secretion is a well-known feature of Klinefelter syndrome in adult males (1). It is generally accompanied by a moderate increase of LH. A preliminary study by Sorensen *et al.* (8) showed that testosterone in cord blood from three neonates with 47,XXY karyotype was slightly lower than in three controls with normal karyotype. We report here that in infants bearing the 47,XXY karyotype, testosterone exhibited a physiological increase
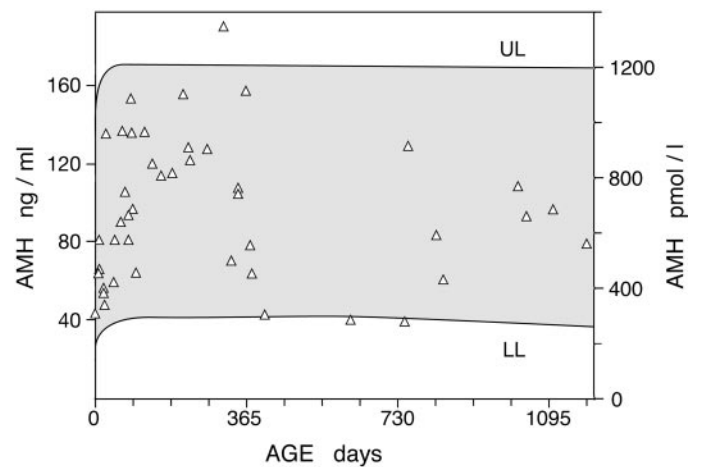
during the first trimester, but the mean level was lower than in normal infants. By contrast, LH levels were similar to those in normal infants, which might denote some degree of resistance to LH, related to the abnormal Leydig cell karyotype, because SHBG levels did not differ from controls. On the other hand, we found a significant correlation between testosterone and LH levels, similar to that previously reported by our group in normal infants (9). This observation raises the question of possible consequences of the blunted neonatal testosterone peak on the adult reproductive axis. Suppressing the neonatal testosterone peak in rhesus monkeys by administration of a LHRH agonist delays puberty; impairs testis development and secretion; induces oligozoospermia or azoospermia (10); suppresses the sexual activity when adult (11); and decreases the hypothalamic sensitivity to the neuromediator amino acid glutamate (12), which plays a key role in the onset of puberty (13). Nevertheless, pubertal development in adolescents with Klinefelter syndrome seems to occur at the appropriate time (14). In addition, it has also been shown in monkeys that treatment with a LHRH antagonist during the neonatal period lowers the social rank of adults (15). Although there have been no systematic evaluations of the social rank in Klinefelter subjects, it should be noticed that mental retardation has been repeatedly reported in recent literature.

In contrast to the impairment in Leydig cell secretion, INHB and AMH secretions are normal in 47,XXY infants.

Inhibin B levels are normal and clearly dependent on FSH

In normal male infants, high serum levels of INHB have been reported by several investigators (16–18). Similar data were obtained in our control group. Interestingly, male infants with nonmosaic 47,XXY karyotype have normal INHB secretion all along the observation period. Inhibin secretion is highly sensitive to FSH, as in normal males. There appears to be a similar strong correlation between INHB and FSH in XXY and control infants, and additionally in a boy, transiently treated with testosterone, INHB and FSH changes were also strictly parallel. This is consistent with the increase

of INHB levels observed by Main *et al.* (19) in an infant treated with FSH for hypogonadotropic hypogonadism.

Conversely in 10 infants with normal karyotype treated with human chorionic gonadotropin (hCG) for 1 wk (1500 IU hCG im every other day), INHB levels fell from 200 ± 28 to 95 ± 17 pg/ml, whereas FSH levels decreased to levels close to the detection limit (our unpublished personal data).

This indicates that inhibin secretion by testis cells, bearing this sex chromosome polysomy, is not impaired in young prepubertal boys, in contrast to adolescents and adults. Whether inhibin secretion in XXY infants results exclusively from Sertoli cells remains to be determined. It should be noticed, however, that immunolocalization studies only in adult testes support the assumption of a significant contribution of germ cells to INHB production (16, 20).

AMH secretion, although not correlated with FSH, is dependent on the trophic effect of FSH on Sertoli cells

AMH secretion is normal in infants with 47,XXY karyotype. AMH levels increased progressively from the first month of life through 6 months of age as in normal infants (21). AMH levels are not correlated with FSH, and the reverse relationship with testosterone, reported by our group in adolescent males (22), was not found here in neither Klinefelter nor control infants. By contrast and despite the different temporal changes, we found a significant correlation between AMH and INHB levels, previously unreported. It has been shown that FSH increased the proportion of proliferating Sertoli cells in cultured rat testis fragments (23). In FSH-deficient transgenic mice, AMH levels and Sertoli cell number are decreased but restored by administration of recombinant FSH (24). Moreover, in primates several reports from Ramaswamy *et al.* (25) and Simorangkir *et al.* (26) have emphasized the effect of FSH on Sertoli cell proliferation. Therefore, is it likely that the correlation between AMH and INHB reflects the trophic effect of FSH on Sertoli cells, rather than direct dependency of AMH on FSH. Furthermore, AMH secretion remained high beyond the age of 1 yr, whereas FSH levels were strongly suppressed, suggesting that once initiated, AMH secretion by Sertoli cells does not require significant FSH stimulation. In addition, as shown in Fig 3, the sharp decrease in FSH does not result in any decrease of AMH. Although anecdotic, this observation emphasizes the complexity of the mechanism regulating AMH secretion, which is still controversial. The reverse relationship with testosterone reported previously (22) is evident in boys treated for precocious puberty with a GnRH agonist: AMH levels significantly increased after testosterone suppression and are decreased after resumption of testis secretion (our personal unpublished data). On the other hand, short-term stimulation of testosterone secretion by hCG in prepubertal boys does not alter AMH (our unpublished data).

Conclusion

There is only scarce knowledge of testicular histology in prepubertal boys with Klinefelter syndrome. In three boys younger than 2 yr old, Mikamo *et al.* (27) found germ cells of normal appearance but reduced number and Sertoli cells with both normal appearance and number. Müller *et al.* (28)

found normal seminiferous tubules, as far as germ cells were concerned, in 11 prepubertal boys. However, information regarding the status of Sertoli cells was lacking in the latter study. Our findings suggest that Sertoli cell functions are qualitatively and quantitatively normal in 47,XXY infants, which is in agreement with the early histological study by Mikamo *et al.* (27).

The role of germ cells in the regulation of Sertoli cell secretion is controversial. INHB secretion has been suggested to be up-regulated and AMH secretion to be down-regulated by germ cells (16, 29). The degeneration of germ cells after puberty could therefore impair INHB and AMH secretions. However, in nonmosaic Klinefelter syndrome, all Sertoli cells, when present in adult testis, have 47,XXY karyotype (30), and that could be enough to induce degeneration. On the other hand, in some subjects 47,XXY spermatogonia can undergo meiosis and give rise to X, Y, XX, and XY spermatozoa (30, 31). These findings have been challenged by other investigators who reported that only spermatogonia with a normal chromosomal pattern can produce mature sperm cells (32). However, both the successful intracytoplasmic sperm injection treatment of infertility in men with Klinefelter syndrome and the present evidence of normal Sertoli cell secretions in infants support the importance of elucidating the mechanism of secondary Sertoli and germ cell degeneration in adolescents with Klinefelter syndrome.

In addition, because there is experimental evidence in primates that testosterone secretion in the neonatal period can be important for adult sexual competence, the benefit of compensatory testosterone administration in Klinefelter infants during the first trimester of postnatal life deserves consideration.

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