Minipuberty in Male Full-term Neonates Appropriate and Small for Gestational Age and in Preterm Babies: Data From a Single Centre

Boncompagni A et al. Assessment of Minipuberty in Male Neonates

Alessandra Boncompagni1, Elisa Pietrella2, Erica Passini2, Chiara Grisolia1, Mara Tagliazucchi1, Enrico Tagliafico1, Licia Lugli1, Alberto Berardi1, Lorenzo Iughetti2, Laura Luccacioni2

1Neonatal Intensive Care Unit, Department of Medical and Surgical Sciences of Mothers, Children and Adults, University of Modena and Reggio Emilia, 41124 Modena, Italy
2Paediatric Unit, Department of Medical and Surgical Sciences of Mothers, Children and Adults, University of Modena and Reggio Emilia, 41124 Modena, Italy

What is already known on this topic?

Previous studies have shown HPG axis activation during early life, also referred to as “minipuberty”. This period has sex-related differences in levels and duration of both gonadotropins and sex steroid’s secretion. The role of minipuberty seems to be significant mainly for development and maturation of reproductive organs, particularly in males.

What this study adds?

This study provides a longitudinal analysis with samples and a between-group comparison in term and preterm infants. This analysis adds valuable information on the postnatal HPG axis activation depending on birth weight and prematurity.

Abstract

Objective: The postnatal activation of the hypothalamic-pituitary-gonadal (HPG) axis is usually known as “minipuberty”. There are still open questions on its biological activity and significance depending on sex, gestational age (GA) and birth weight (BW) with few longitudinal data.

Methods: Single-centre longitudinal study to quantify urinary FSH (uFSH), LH (uLH) and testosterone (uTs) in male neonates. 46 neonates were enrolled and sorted into 3 subgroups: 23 full-term boys appropriate for GA (FT AGA), 11 full-term boys with BW ≤ 3rd centile (FT SGA), and 12 preterm (PT) boys ≤ 33 weeks of GA. Urinary hormones were measured with electrochemiluminescence immunoassay and correlated to simultaneous auxological parameters, linear growth and external genitalia at scheduled time-points.

Results: PT boys display a pulsatile pattern of urinary gonadotropins (uGns) with higher levels of uLH and a gradual increase of uTs. Testicular descent starts from 29–32 weeks with the peak of uTs. During the first 12-months post term age (PTA), FT AGA boys display a better linear growth (p < 0.05). PT show higher uGns levels until 3-months PTA. Considering chronological age, PT babies in the first 90 days of life have higher uLH levels than FT AGA with a peak at 7 and 30 days (p < 0.001) and higher uTs levels. Correlation analysis between penile growth of all neonates and uTs is significant (p = 0.04) but not when subgrouping.

Conclusions: This study provides valuable information on the postnatal HPG axis activation in term and preterm infants. Minipuberty may involve an early window of opportunity to evaluate HPG axis functionality. Further studies with a long-term follow-up are needed with a special focus on possible consequences of GA and BW.

Keywords: Minipuberty, urinary gonadotropins, newborn, infants, prematurity, growth

Corresponding author: Alessandra Boncompagni MD
Neonatal Intensive Care Unit, Department of Medical and Surgical Sciences of Mothers, Children and Adults, University of Modena and Reggio Emilia, 41124 Modena, Italy
+0594222522
boncompagni.alessandra@aou.mo.it
30.05.2023
17.09.2023

Published date: 26.09.2023

Introduction

Puberty is preceded by two periods marked with a transitional activation of the hypothalamic-pituitary-gonadal (HPG) axis: the first during foetal life, playing a crucial role in sex determination, the second during first postnatal months. Such HPG axis activation during early life is also referred to as “minipuberty” (1), and its biological significance is still not completely understood.

Studies on moderate cohorts of neonates have shown that minipuberty has sex-related differences (2) in levels and duration of both gonadotropins and sex steroid’s secretion. The gonadotropins start rising at around 1 week of age, peak reaching pubertal values between 1 and 3-months of life, and decline to prepubertal values towards the age of 6 months (3–7). Male neonates have a Luteinizing Hormone (LH) peak higher than Follicle Stimulating Hormone (FSH) levels with a gradual decrease to prepubertal levels at around 6–9 months (3,6). Testosterone (T) level starts to increase following the LH rising with similar pattern (5,6,8). Both LH and T reach higher levels in boys than in girls (3–10). On the contrary, female infants have predominant FSH levels that remain elevated for a longer period whereas estradiol (E) levels display a fluctuant pattern, probably reflecting the ovarian follicular cycle of growth and atrophy (1).

The role of minipuberty seems to be significant mainly for development and maturation of reproductive organs, particularly in males (11). In addition, androgens have been related also to other aspects of infant growth such as cutaneous manifestations (12,13), linear growth, bone mineralization, psychosexual development and behaviour (14–25).

Little is known on the influence of, e.g., birthweight or prematurity on the HPG axis. The HPG axis activation in babies born small for gestational age (SGA) is not well defined and its short-term and long-term effects on growth and development are still controversial. Studies on SGA females have found higher postnatal FSH levels compared with neonates born appropriate for gestational age (AGA). Meanwhile, in male SGA term neonates, HPG axis activation has been linked both to lower (26) and higher (27) gonadotropins and androgens (28) levels with uncertain effects in adult life (29). Also, the impact of prematurity has been investigated mainly with cross-sectional studies based on serum
samples (3, 4, 6) but only a few adopted a longitudinal approach. Based on recent longitudinal studies using urinary gonadotropins (uGns) (1), preterm birth does not seem to influence the onset of postnatal HPG axis activation, as gonadotropin levels begin to rise with the same timing on full-term (FT) and preterm (PT) infants. Moreover, minipuberty in PT babies seems to be stronger and prolonged with uncertain significance and effects (30–32) but data are still not univocal. Understanding the physiological trend of hormonal levels during minipuberty and its differences between term, preterm and SGA boys is therefore necessary to provide a better comprehension of all possible short-term and long-term effects.

Aims of this study are to quantify urinary FSH (uFSH), urinary LH (uLH) and urinary testosterone (uTs) in male neonates, and to compare changes in timing and magnitude of uGns (uFSH, uLH) and uTs secretion in 3 different groups of neonates: full-term boys appropriate for gestational age (GA) (FT AGA), full-term boys small for GA (FT SGA), PT boys ≤33 weeks of GA. Secondary outcomes are between-group comparisons of external genitalia clinical examination (penile length, testicular volume and position) and linear growth, with attention to the catch-up growth of FT SGA and PT infants.

**MATERIALS AND METHODS**

**Population**

This is a single-centre longitudinal and prospective study conducted on male neonates. Neonates enrolled were split into 3 categories: FT AGA boys, FT SGA boys and PT boys born before 33 weeks of GA. Comparisons were made according to calendar age or post-term age (PTA). Neonates with antenatal genetic diagnosis or severe metabolic, cardiovascular, endocrine or sex development disorders (33) were excluded from enrolment.

Being classified as AGA or SGA among FT boys depended on birth weight (BW) (34). SGA was considered as a boy with BW <3rd centile whereas all FT AGA boys had a BW >10th centile.

For PT babies, no classification according to BW was performed. Medical data were collected from clinical records. Clinical assessments for full-term neonates were: within the first 72 hours of life, at 1 week, at 1.5–6 months of life. For preterm neonates evaluations were: within first 72 hours of life, once a week until term age, at 1.5–6 months of PTA. Number of evaluations depended on GA at birth and the child’s clinical conditions.

At each scheduled time-point, both clinical evaluation and urinary collection were performed. Clinical evaluation included auxological parameters (weight, length, head circumference and external genitalia) and endocrine tests.

Weight was measured with a digital baby scale to the nearest 5 g. The recumbent length was measured with a portable high quality infantometer (GIMA, Baby Height Measuring Mat) to the nearest 0.1 cm. HC was measured using a measuring tape from the most prominent part of the forehead around to the widest part of the back of the head. If the baby’s condition did not allow measurements among preterm babies in the Neonatal Intensive Care Unit (NICU), both clinical evaluation and urine collection were postponed.

Weight and birth weight and the following growth till term age was monitored using the national charts (34). For term babies, being AGA or SGA was assessed according to national charts (34). The international longitudinal charts WHO 2006 (35) were used from 1- to 12-months PTA. National charts at birth and within term corrected age was preferred due to the fact that our study is a single centre study. National charts within the first two years PTA are not available.

Penile length was measured as described by Boas M et al. (11) and reported as a numeric value. The penis was slightly straightened and the distance between the lower edge of the pubic bone and the tip of the shrunken foreskin (excluding foreskin) was measured using a caliper.

All measurements were assessed by trainees in Paediatrics and repeated two times to reduce errors.

Testicular position was classified according to Boisen KA et al. (36) as “non-palpable”, “inguinal” or “descended”. Testicular volume was quantified using the Prader orchidometer.

**Urine collection and assays**

Urine was collected using a plastic bag made to fit baby’s genital area. Urine collection and assays were performed on fresh urine with regards to FSH, LH, uTs and urinary creatinine (uCr). All measurements were assessed by trainees in Paediatrics and repeated two times to reduce errors.

The average uTs during first 3 months of age was calculated using the area under the curve (AUC) with the trapezoid al rule and divided for the total time. Subsequently, the average hormone level was correlated with the penile curve (AUC) with the trapezoidal rule and divided for the total time. Subsequently, the average hormone level was correlated with the penile curve (AUC) with the trapezoidal rule and divided for the total time. Subsequently, the average hormone level was correlated with the penile curve (AUC) with the trapezoidal rule and divided for the total time.
Comparison between groups depending on PTA

Auxological parameters and external genitalia

Auxological parameters and penile length are shown in Table 1. The between-group comparison is shown in Figure 1. At birth or at expected term for PT infants) weight was higher in FT AGA neonates compared to FT SGA(p=0.001) and PT(p=0.001). This difference remained significant at 1-3-6 months PTA only between FT AGA and FT SGA babies(respectively p=0.001, p=0.013, p=0.001). Moreover, no differences between FT SGA and PT boys were observed at any time-point.

Length percentile was higher in FT AGA boys from birth to 6-months PTA, both when compared to FT SGA(p<0.001) and to PT babies(p=0.001).

No differences in penile length were found apart from a slightly divergence at 1-month PTA in which FT AGA and FT SGA had longer measurements in comparison with PT boys(p=0.03). Testicular volume did not show any difference.

Urinary gonadotropins and urinary testosterone

Longitudinal trend of hormonal levels until 12-months PTA are shown in Figure 2. uFSH and uLH were significantly higher at the expected term in PT neonates in comparison with FT SGA(p=0.054 and p=0.021, respectively). Comparison between PT boys and FT AGA display higher level of uLH at expected term and 3-months PTA(p=0.001 and p=0.026, respectively). On the contrary, uTs was significantly higher in FT AGA neonates both in comparison with FT SGA and PT babies(p<0.001).

Linear growth, external genitalia development and hormone levels in preterm infants

Longitudinal trend of linear growth in preterm boys from birth to discharge showed a significant decrease during the hospital stay(p<0.05).

Looking at the individual values of uGns and uTs, a great variability was observed among PT male neonates. Both uGns and uLH displayed a pulsatile pattern starting within the first 4 weeks of life. Subsequently, also uTs values had a rise over this period. However, individual trend of duration and magnitude of this HPG axis activation over the first 2 months of life was greatly variable between different subjects. Average levels of uFSH, uLH had a pulsatile pattern with higher values of uLH, as expected in male neonates, uTs is high at birth with a subsequent decrease and a second rise just after the uLH peak at 3-4 weeks of life. Thereafter uTs levels remained high and testicular descent of PT babies was parallel to uTs surge. Individual and mean values of urinary hormone levels are shown in Figure 3.

As shown in Figure 4, PT babies revealed a postnatal HPG axis activation that is prolonged in its duration but also in its amplitude in comparison with both groups of term neonates(AGA and SGA).

Comparison between groups depending on calendar age

External genitalia

Penile length was higher in FT AGA and FT SGA neonates at birth, at one week and at 30 days of life(p<0.001) compared to PT boys. Penile length was higher in SGA neonates at 90 days(p=0.028)

Urinary gonadotropins and urinary testosterone

The mixed model analysis revealed a very different pattern in longitudinal assessment of urinary hormone levels between groups using calendar age during the first 3 months of life.

At birth, uTs was higher in FT AGA neonates compared to PT and FT SGA babies(p=0.001 and p=0.001, respectively). At 7 days of life, uLH had a peak at higher levels in PT boys compared to FT AGA(p=0.004). At 30 days PT male neonates continue to have higher levels of uLH both compared to FT AGA and FT SGA(p=0.001) and PT babies display also higher uTs levels in comparison with FT AGA boys(p=0.05).

At 90 days of life PT boys still had higher levels of uTs both in comparison with FT AGA and SGA male babies(p=0.015 and p=0.05, respectively) as in Figure 5.

Correlation analyses

To test the hypothesis that minipuberty correlates with external genitalia development and linear growth, a correlation analysis was performed between uTs levels and auxological parameters (recumbent length and penile length), considering the calendar age.

No significant differences were found between uTs and linear growth within the first 3 months; on the contrary, a significant correlation was found between uTs and penile growth(rho=0.412, p=0.04).

DISCUSSION

The development of highly sensitive immunohistochemical and chemiluminesometric assays for measurement of gonadotropin levels in clinical research studies highlighted a strong correlation between serum and urine samples(37–39). Despite this, there are still no validated reference values for uGns in children according to sex, age and pubertal stage that allow their use in routine clinical settings. However, all studies using urine samples to investigate the HPG axis agree on their use in clinical research settings(18,40–42).

At birth uGns are low with a subsequent gradual increase and a peak between 1 week and 3 months, with uLH levels predominant over uFSHs. Our longitudinal study on male infants allowed the identification of temporal relationships in hormonal levels and their effects on catch-up growth and external genitalia development. Moreover, splitting neonates into 3 categories allowed us to better elucidate the influence of birthweight and prematurity on this post-natal HPG axis activation.

In fact, the influence of prematurity on sexual hormone levels has been investigated by few studies, mainly cross-sectional and with slightly different findings(30–32). The only one study with a longitudinal design(7) found higher uLH and uTs levels in preterm boys as well as an increase of testosterone levels in all neonates with a peak at 1 month and a positive correlation with penile growth. Even the impact of being SGA on the postnatal HPG axis activation is still not elucidated(26,27,29,43). Data are not univocal and the definition itself of a SGA neonate embraced multiple possible criteria(44,45).

Our results are in line with the longitudinal study by Kuiri-Hänninen T et al(7). PT boys have higher uLH and uTs levels from one week to 3 months of life, even if this difference is not always significant. uLH levels at 7 days and 1 month were higher also in FT SGA neonates than in FT AGA. uTs levels were higher in PT boys with a postnatal rise of testosterone at around 1 month in all neonates. uFSH has a higher peak at one week in FT SGA neonates but a subsequent decline towards one month of age. On the contrary, uTs in PT babies peaked later but lasted longer with higher levels at 3 months in comparison with FT AGA boys. Therefore, preterm birth seems to have a great influence both on magnitude and duration of this postnatal HPG axis activation.

However, comparing neonates depending on PTA, it is interesting to notice that differences between FT and PT boys are gradually reduced with still higher uGns in PT at the expected term of gestation but no differences at 6- and 12-months PTA.

These findings support the idea that the postnatal HPG axis activation is developmentally regulated(1). The postnatal pituitary activation becomes evident at a similar time for PT infants and persists longer and higher in preterm babies. PT boys thus probably experience a maturation of the hypothalamic feedback mechanisms as term approaches.

Postnatal HPG axis activation has been suggested to play a role also in completing the external genitalia development(18,40,41). We found a significant correlation between uTs levels and penile growth. However, even if PT boys displayed higher levels of uTs, penile growth of PT boys was not significantly different from FT AGA and FT SGA male neonates, in contrast with previous studies(7,11). Penile length of PT boys showed a catch-up growth during the first months of life leading to no significant differences at 3-6 months of age. Moreover, testosterone surge and testicular descent were strongly related in PT infants.

Coming to the effects of testosterone levels on catch-up growth: in contrast with previous studies(18) no significant correlation between uTs and growth velocity was found, but the recumbent length at 6-months PTA had no differences between groups, suggesting a catch-up growth
of FT SGA and PT boys. Strength of our study is related to the age of our population. First, we performed a longitudinal analysis with serial samples and a between-group comparison that allows a better analysis of the influence of birth weight and prematurity on the HPG axis. It is our knowledge that the present study is the only one to investigate this axis only in extremely and moderately preterm infants. In parallel, we did not include neonates with a weight between 20th and 90th percentile, to not overlap with other studies and to better appreciate the influence of low birth weight on this axis.

**Study limitations**

Some limitations need to be highlighted. First, the sample size is limited. Recruitment of preterm babies can be challenging because of their clinical conditions and their lower numerosity. Concurrently, the enrolment of healthy infants in a prospective study until 12-months of age can be difficult for families' compliance. We did not perform a correlation analysis with serum samples, but the use of uGns in research has been largely developed, making us confident that our results can reflect the pattern of serum gonadotropins and improving the family compliance. However, it is important to underline how the methodology of this study could have been empowered by the comparison between uGns and serum gonadotrophins and salivary testosterone, especially in preterm babies due to the immaturity of the tubular activity.

The need for creatinine correction during the neonatal period is still controversial, especially in neonates (37–40,46,47). Creatinine clearance is low at birth and the rate is positively correlated with weight, length and post-conceptional age, but negatively correlated with gestational age (48). This information can explain our lower Ucr values in premature infants and it is probably the reason of the between groups significant difference. We can hypothesize to privilege the use of uGns not-corrected for creatinine excretion in a comparison between full-term and preterm neonates but this suggestion needs to be interpreted with caution. uTs levels were assessed with a chemiluminescent radioimmunoassay, not with mass spectrometry (MS). This can be relevant not only to exclude the influence of other androgens, but mainly to detect the low levels after the peak of testosterone at 1-3 months. In fact, our results considering PTA revealed low to undetectable uTs levels just after 3 months whereas other studies on serum and urine samples displayed higher levels in male infants until 6-9 months PTA. However the use of an immunoenzymatic method for testosterone detection is in line also with other studies that used urine samples (12,18).

**CONCLUSIONS**

Our study provided insight on the postnatal HPG axis activation in full-term AGA and SGA boys, as well as in preterm infants. Results suggest that minipuberty is increased and prolonged in PT boys in comparison with the previous full-term babies only when the analysis depends on calendar age, suggesting an ontogenetic regulation. The enhanced activation of the HPG axis translates in a more pronounced androgenic secretion with a faster penile growth in PT neonates during the first 3 months of age. On the contrary, results in FT SGA infants are difficult to be interpreted and need a sample size extension. SGA neonates had lowest postnatal testosterone levels even if with higher uGns levels and a same trend of penile growth possibly reflecting a different intrauterine stimulation due to a growth restriction. However, the lack in postnatal peak of testosterone translated in a slower catch-up growth in FT SGA neonates. In conclusion, minipuberty provides an important window of opportunity for the evaluation of the HPG axis functionality before puberty. uGns have been demonstrated to be a valid, practical and non-invasive tool for the purpose, an enforcement of this method among infants would be favorable. The possible short-term and long-term implications of this different postnatal activity in SGA or preterm neonates need to be clarified. Further studies with a long-term follow-up are needed with regards to infants and also depending on birthweight and prematurity.

**Acknowledgments:** We want to thank all the neonatal nurses and pediatric trainees of the University Hospital of Modena for their help with samples' collection. Many thanks also to Prof. Faisal Ahmed and Dr. Martina Rodie from the Developmental Endocrinology Research Group of Glasgow University for the remote support.

**Authors contributions:** Surgical and Medical Practice: Alessandra Boncompagni, Elisabetta Pietrella, Erica Passini, Lucia Lugli, Alberto Berardi, Lorenzo Iughetti, Laura Lucaccioni, Concept: Alessandra Boncompagni, Laura Lucaccioni, Design: Alessandra Boncompagni, Laura Lucaccioni, Data Collection or Processing: Alessandra Boncompagni, Elisabetta Pietrella, Erica Passini, Chiara Grisolia, Mara Tagliazucchi, Enrico Tagliafico, Analysis or Interpretation: Alessandra Boncompagni, Chiara Grisolia, Mara Tagliazucchi, Enrico Tagliafico, Lorenzo Iughetti, Laura Lucaccioni, Literature Search: Alessandra Boncompagni, Lucia Lugli, Alberto Berardi, Lorenzo Iughetti, Laura Lucaccioni, Writing: Alessandra Boncompagni, Elisabetta Pietrella, Lucia Lugli, Alberto Berardi, Lorenzo Iughetti, Laura Lucaccioni.

**Financial Disclosure:** This research project was supported with a research grant of the University of Modena and Reggio Emilia for the Department of Clinical and Surgical Sciences for Mothers, Children and Adults at the University Hospital of Modena (FAR 2017).

**REFERENCES**


Supplementary Table 1. Maternal and Obstetrics data of all neonates included in the study.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>FT AGA (n=23)</th>
<th>FT SGA (n=11)</th>
<th>PT (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational Diabetes</td>
<td>1 (2,1%)</td>
<td>1 (9,1%)</td>
<td>1 (2,1%)</td>
</tr>
<tr>
<td>Thyroid Disorders</td>
<td>/</td>
<td>1 (2,2%)</td>
<td>/</td>
</tr>
<tr>
<td>Hypertension/Preeclampsia</td>
<td>/</td>
<td>/</td>
<td>4 (8,7%)</td>
</tr>
<tr>
<td>Other metabolic impairments</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>None</td>
<td>22 (47,8%)</td>
<td>9 (19,5%)</td>
<td>7 (15,2%)</td>
</tr>
<tr>
<td>Antenatal Ultrasound</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal abnormalities</td>
<td>1 (2,2%)</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Severe cardiac abnormalities</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Others</td>
<td>1 (2,2%)</td>
<td>1 (2,2%)</td>
<td>/</td>
</tr>
<tr>
<td>None</td>
<td>21 (45,6%)</td>
<td>10 (21,7%)</td>
<td>12 (26,1%)</td>
</tr>
<tr>
<td>Maternal Serology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive for TORCH</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Negative for congenital infections</td>
<td>23 (50%)</td>
<td>11 (23,9%)</td>
<td>12 (26,1%)</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>19 (41,3%)</td>
<td>11 (23,9%)</td>
<td>4 (8,7%)</td>
</tr>
<tr>
<td>Elective/Emergency Caesarean Section</td>
<td>4 (8,7%)</td>
<td>/</td>
<td>8 (17,4%)</td>
</tr>
<tr>
<td>Infective risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vagino-rectal swab GBS positive or unknown</td>
<td>1 (2,2%)</td>
<td>2 (4,3%)</td>
<td>10 (21,7%)</td>
</tr>
<tr>
<td>Previous children with GBS neonatal infection</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Incomplete intrapartum chemoprophylaxis</td>
<td>/</td>
<td>/</td>
<td>2 (4,3%)</td>
</tr>
<tr>
<td>Antenatal Corticosteroids (% calculated only for PT babies)</td>
<td>Complete</td>
<td>/</td>
<td>8 (66,7%)</td>
</tr>
<tr>
<td>Incomplete/Not done</td>
<td>/</td>
<td>/</td>
<td>4 (33,3%)</td>
</tr>
</tbody>
</table>
# Supplementary Table 2. Characteristics of the study population.

Data are expressed as mean (range) for continuous variables and as number (% within group) for categorical data.

<table>
<thead>
<tr>
<th></th>
<th>FT AGA (n=23)</th>
<th>FT SGA (n=11)</th>
<th>PT (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GA (weeks, days)</strong></td>
<td>39.3 (38-41.3)</td>
<td>39.1 (37-40.3)</td>
<td>29.6 (25.6-33)</td>
</tr>
<tr>
<td><strong>BW (gr)</strong></td>
<td>3488 (2990-4150)</td>
<td>2457 (2180-2770)</td>
<td>1245 (850-1830)</td>
</tr>
<tr>
<td><strong>BW (centile)</strong></td>
<td>58.9 (19-96)</td>
<td>2 (0-3)</td>
<td>47.1 (6-95)</td>
</tr>
<tr>
<td><strong>Length (cm)</strong></td>
<td>50.8 (48-54)</td>
<td>47.5 (45-50)</td>
<td>37.2 (34-61)</td>
</tr>
<tr>
<td><strong>Length (centile)</strong></td>
<td>59.7 (11-99)</td>
<td>14 (1-46)</td>
<td>35 (1-84)</td>
</tr>
<tr>
<td><strong>HC (cm)</strong></td>
<td>34.7 (32-37)</td>
<td>33.1 (30-36)</td>
<td>34.3 (24-31)</td>
</tr>
<tr>
<td><strong>HC (centile)</strong></td>
<td>55.3 (4-97)</td>
<td>27.5 (0-89)</td>
<td>47.3 (2-98)</td>
</tr>
<tr>
<td><strong>AI 1 minute</strong></td>
<td>8.7 (6-10)</td>
<td>9 (8-10)</td>
<td>6.5 (2-8)</td>
</tr>
<tr>
<td><strong>AI 5 minutes</strong></td>
<td>9.9 (9-10)</td>
<td>9.8 (9-10)</td>
<td>8.4 (7-9)</td>
</tr>
<tr>
<td><strong>AI 10 minutes</strong></td>
<td>NA</td>
<td>NA</td>
<td>8.2 (8-9)</td>
</tr>
<tr>
<td><strong>Days of mechanical ventilation</strong></td>
<td>NA</td>
<td>NA</td>
<td>4 (0-20)</td>
</tr>
<tr>
<td><strong>Days of non-invasive ventilation</strong></td>
<td>NA</td>
<td>NA</td>
<td>24.5 (1-60)</td>
</tr>
<tr>
<td><strong>Days of oxygen therapy</strong></td>
<td>NA</td>
<td>NA</td>
<td>31.9 (0-70)</td>
</tr>
<tr>
<td><strong>Respiratory distress</strong></td>
<td>2 (8.7%)</td>
<td>0</td>
<td>10 (83.3%)</td>
</tr>
<tr>
<td><strong>Phototherapy for jaundice</strong></td>
<td>1 (4.3%)</td>
<td>1 (9%)</td>
<td>10 (83.3%)</td>
</tr>
<tr>
<td><strong>Hypoglycaemia</strong></td>
<td>0</td>
<td>1 (9%)</td>
<td>10 (83.3%)</td>
</tr>
<tr>
<td><strong>Hypospadias</strong></td>
<td>0</td>
<td>0</td>
<td>2 (16.7%)</td>
</tr>
</tbody>
</table>

Abbreviations: AI: Apgar index; BW: birth weight; GA: gestational age; HC: head circumference; NA: not assessed; SDs: standard deviations. 
*Both cases had anterior hypospadias without other genital abnormalities. The penis length and shape was not altered by the meatus position, due to the mildness of the hypospadias grade. No cases with middle or posterior hypospadias were included in the study to avoid possible presence of DSD cases among the study population.
Figure 1 Linear trend of auxological and external genitalia parameters from birth (corresponding to the presumed term of GA for PT boys) to 12 months of post-term age (PTA). Neonates were divided into 3 categories: FT AGA (blue), FT SGA (red), PT (green). Differences statistically significant are indicated with this legend: * FT AGA ≠ PT, ● FT AGA ≠ FT SGA, ◊ FT SGA ≠ PT.
Fig. 2 Trend of hormone levels from birth (expected term of GA for PT boys) until 12 months of post-term age (PTA). Neonates were divided into 3 categories: FT AGA (blue), FT SGA (red), PT (green). Urine samples were assessed for uFSH (A), uLH (B), uTs (C). Differences statistically significant are indicated with this legend: * FT AGA$\neq$ PT, ♦ FT AGA$\neq$ FT SGA, ◊ FT SGA$\neq$ PT.
Fig. 3 Trends of hormone levels during first weeks of life among PT babies. First three graphs show individual pattern of each premature boy using a different colour for each subject. The graph at the bottom of this figure shows the mean trend of urinary hormone levels of all PT boys.
Fig. 4 Effects of preterm birth on levels of uFSH, uLH and uTs. Trend of preterm male neonates is compared with full-term neonates until 52 weeks of CGA.
Fig. 5 Longitudinal assessment of hormone levels from birth to 3 months of life using calendar age. Neonates were divided into 3 categories: FT AGA (blue), FT SGA (red), PT (green).

Urine samples were assessed for uFSH (A), uLH (B), uTs (C). Differences statistically significant are indicated with this legend: * FT AGA≠ PT, ♦ FT AGA≠ FT SGA, ◊ FT SGA≠PT.