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The Sertoli cell hormones inhibin-B and anti Müllerian hormone have different patterns of secretion in prepubertal cryptorchid boys

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ABSTRACT

Objectives and hypotheses: The Sertoli-cells produce inhibin-B and Anti-Müllerian-Hormone (AMH). Much is still unknown about these hormones in prepubertal cryptorchids. The Sertoli-cells are mandatory for germ cell development. The aim of the study was to investigate if there are differences in secretion pattern of Sertoli-cell hormones and their gonadotropin feed-back mechanisms.
Methods: Included were 94 prepubertal cryptorchid boys 0.5–13.1 years with measurements of serum-inhibin-B, Anti-Müllerian-Hormone (AMH), Luteinizing Hormone (LH) and Follicle Stimulation Hormone (FSH). The serum values were measured using commercially available kits. The hormonal values were related to age-matched normal values. Testicular biopsy was taken at orchiopexy.
Results: Inhibin-B positively correlated to AMH for 1–13 year-old patients ($p < 0.0001$), but not for 0.5–1 year-old patients ($p = 0.439$). For 0.5–1 year-old patients inhibin-B-values tended to decrease ($p = 0.055$), in contrast to AMH-values ($p = 0.852$).
LH was elevated more often than FSH ($p = 0.014$). FSH and LH were positively associated in patients both 0.5–1 year ($p = 0.042$) and 1–13 years of age ($p < 0.0001$). LH correlated positively to inhibin- B ($p = 0.001$). In contrast, FSH did not correlate to inhibin-B or AMH ($p = 0.755$ and $p = 0.528$). The number of A-dark spermatogonia per tubular transverse section was positively correlated to inhibin-B serum level.
Conclusion: Our new finding of an association between LH and inhibin-B in infancy of cryptorchid boys may be essential for the transformation of gonocytes to A-dark spermatogonia. Previously, LH associated to inhibin-B was described in early puberty only. During the first year of life inhibin-B values decreased faster than AMH. The AMH-levels may just reflect the increased Sertoli cell number that occurs during the first 3 months of life.

The Sertoli cells produce inhibin-B and Anti-Müllerian Hormone (AMH). During the first months of life the hypothalamic–pituitary–gonadal hormone axis is transiently activated resulting in increased serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, inhibin-B and AMH.

The inhibin-B has a maximum value at around 3 months of age [1], and the elevated inhibin-B persists for a longer period of time than the elevated FSH, LH and testosterone, as it remains elevated to 15 months of age and decreases to basal levels during the third year of life [1,2]. During the normal prepubertal period inhibin-B is secreted in significant amounts, while FSH and testosterone are low. In puberty the inhibin-B levels increase to reach adult levels, which are somewhat lower than the values at 3 months of age [1–3].

There are different reports about the values of the AMH levels. It has been reported that the AMH exhibits a peak value at 3 months of age, declines at 12 months and remains at a relatively stable low level throughout childhood until puberty, where AMH declines progressively so adults exhibit low levels after puberty [4]. In contrast, AMH has also been reported to show a maximum between four and twelve months of age [5,6], and to exhibit peak values in late infancy [7], and AMH peak levels therefore occur after the postnatal surge of androgen secretion [7]. Prenatally, AMH causes regression of the Müllerian ducts in the male fetus [4–7], and it may be the main function of AMH, as the postnatal impact of AMH is not described with certainty apart from a role in the ovarian cycle in females [8]. During the first 3 months of life there is an increase in the Sertoli cell number [9] and the increased AMH level may just reflect this [10]. In cryptorchid patients Zivkovic and Hadziselimovic [11] also found that the number of Sertoli cells increased with the hormonal surge, but the Sertoli cell number was diminished compared to the normal testis.

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In cryptorchid testes, the serum inhibin-B reflects the state of germinative epithelium, and inhibin-B values have been described to be lower in cryptorchid than in normal boys [12]. When levels of AMH in normal children were compared with those undergoing orchidopexy, a deficient surge of AMH secretion was also seen between 4 and 12 months of age and in later childhood [5]. Other studies have shown that serum AMH is known to be valuable in assessing gonadal function. Several studies have reported lower serum AMH concentrations in boys with cryptorchidism who were compared with their age-matched counterparts with palpable testes [13,14].

To our knowledge, few studies have focused on inhibin-B and AMH in prebubertal cryptorchid boys, in respect to associations with FSH and LH, age, location of the undescended testes and whether there is bilateral or unilateral cryptorchidism. Pierik et al. in 2009 [15] analyzed sera from a population sample of infants with cryptorchidism (n = 43) and controls (n = 113) for inhibin-B, AMH, testosterone, LH, FSH, and sex hormone binding globulin. After age-correction, a negative correlation between FSH and inhibin-B was observed. The only significant group-differences were lower testosterone levels in cryptorchidism cases. We hypothesize that inhibin-B and AMH in prepubertal cryptorchid boys exhibit different patterns, and are associated with LH and FSH in different ways. Our results may illuminate aspects of undescended testes, which may be of importance for understanding the transformation of gonocytes into the Adult dark Spermatogonia, which takes place during the first 12 months of age, a step that is postulated to be crucial for subsequent fertility since stem cells for spermiogenesis are created in this process [16].

1. Material and method

We included 94 of 117 consecutive prepubertal cryptorchid boys, who had blood samples taken immediately before surgery, in the period May 1–December 31 2014 for inhibin-B, AMH, FSH and LH. The inhibin-B values of 27 patients were also included in a previous study [17]. At surgery the undescended testes were classified clinically and the diagnosis of cryptorchidism was confirmed by testicular biopsy.

Excluded were 23 eligible patients seen in the period May 1–December 31 2014 with pubertal development at physical examination (n = 8) or a disorder of sex development (DSD) (n = 2), chromosomal abnormalities (n = 2), bilaterally no testicular parenchyma (n = 1), previous inguinal surgery (n = 1), those who had received hormonal therapy (n = 3) and those with some blood samples missing (n = 6).

The patients were analyzed by age at surgery, up to 1 year of age versus older than 1 year of age, bilateral versus unilateral cases, and in accordance to the location of the testes at surgery.

2. Hormonal assays

Blood samples were obtained by venipuncture between 8:00 am and 11:00 am. Serum samples were separated of the clot by 10 min centrifugation at 2000 ×g. Serum was stored at −80 °C until analysis. Serum inhibin-B levels were measured using a commercially available inhibin-B ELISA kit (Beckman Coulter Inc., Webster, TX, USA), and with a research kit as recommended by the manufacturer's instructions. The lower detection limit was 3 pg/ml, and the measurements were made in duplicate. Normal reference serum levels on inhibin-B were defined as described by Andersson et al. 1998 [1].

Serum AMH levels were measured using a commercial available AMH ELISA kit (Immunotech, Beckman Coulter Ltd., Marseilles, France (A16507)), and with research kit as recommended by the manufactory instructions. The lower detection limit was 2 pmol/l, and the measurements were made in duplicate. Normal reference serum levels on AMH were defined as described by Aksglaede et al. 2010 [4].

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by sandwich electrochemiluminescence-immunoassay (ECLIA) singly, with commercially available reagents from Roche (catalog no. 11732234122 for LH and catalog no. 11775863122 for FSH). The lowest value of FSH and LH to be measured was 0.05 IU/l. Normal reference serum levels of gonadotropins were defined as described by Andersson et al. 1998 [1].

Testicular biopsies: All tissue specimens were fixed in Stieve's solution, embedded in paraffin, and 2-μm sections were stained with hematoxylin–eosin, CD99 (MIC-2) and PLAP. In blinded fashion the number of Adult dark spermatogonia per tubular transverse section was measured from at least 100 tubular transverse sections. For every patient the mean number of Adult dark spermatogonia per tubule (Ad/T) was found. In the hematoxylin–eosin staining the Adult dark Spermatogonia were identified according to the descriptions by Roosen-Runge and Barlow [18] and Huff et al. [19]. They are situated peripherally in the tubules and have fairly large pale cytoplasma. In the nuclei a vacuole is observed which appears in the sections as a circular light area, usually well defined by a fine membrane which is stained with hematoxylin. The contents of the vacuole do not stain with hematoxylin, but can be faintly stained with eosin. The vacuole usually has at least half the nuclear diameter, but occasionally smaller vacuoles are seen (Fig. 1).

3. Statistics

We used Spearman rank, Fisher exact and Kruskal–Wallis tests to assess statistical significance, and two-sided p values less than 0.05 were considered significant. The statistics programs used were the IBM, SPSS 19 and the statistics program Open Source Epidemiologic Statistics for Public Health, http://www.openepi.com/Menu/OE_Menu.htm.

4. Ethics

The study was conducted according to the Helsinki II declaration, and informed consent was obtained from the parents of the patients. Ethical Committee of Copenhagen file number was KF-01299830.

Funding

No funding.

5. Results

There were a total of 94 prepubertal cryptorchid boys included. The hormonal values appear in Fig. 2A–D related to the normal values [1,4].

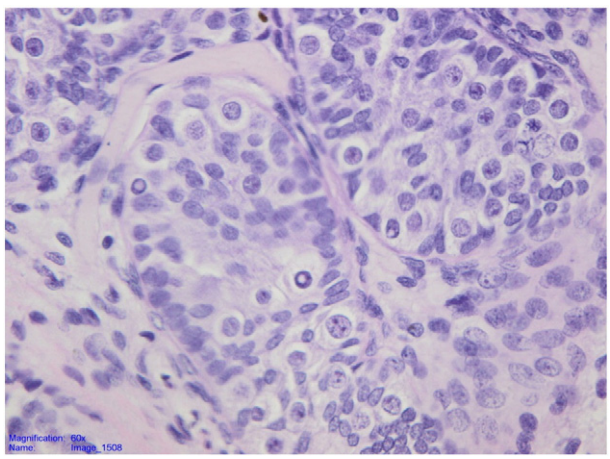

Fig. 1. Hematoxylin + eosin stained testicular biopsy from a 10 month old boy showing presence of Adult dark spermatogonia in the seminiferous tubules. The Adult dark spermatogonia are situated peripherally in the tubules and have pale cytoplasma. In the nuclei a vacuole is observed which appears in the sections as a circular light area.