The Relation between Adult Dark Spermatogonia and Other Parameters of Fertility Potential in Cryptorchid Testes

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Abbreviations and Acronyms

Ad = adult dark

Ad/T = Ad spermatogonia per transverse tubule

FSH = follicle-stimulating hormone

LH = luteinizing hormone

PLAP = placental-like alkaline phosphatase

S/T = germ cells per transverse tubule

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Purpose: The fertility potential of boys with cryptorchidism may be related to the number of adult dark spermatogonia per tubular transverse section in testicular biopsies taken at orchiopexy. Placental-like alkaline phosphatase positive gonocytes in testes within year 1 of life indicate preserved ability for germ cell transformation. We related these parameters to the total number of tubular germ cells and other factors associated with fertility potential.

Materials and Methods: The study comprised 89 boys 0.7 to 3 years old (median age 1.8) who underwent bilateral testicular biopsy at bilateral orchiopexy and provided blood samples for gonadotropins and inhibin B.

Results: Of 76 boys with adult dark spermatogonia 44 (58%) had a normal mean number of spermatogonia per tubular transverse section compared to 2 of 13 (15%) without adult dark spermatogonia ($p < 0.05$). In the 30 boys with good fertility potential, including a normal mean number of tubular germ cells, and normal gonadotropins and inhibin B, the mean number of adult dark tubular germ cells was 0.081 vs 0.031 in the 38 with low fertility potential, including impaired tubular germ cells and/or low inhibin B but no reactive increase in gonadotropins ($p < 0.05$). In the 21 patients with increased gonadotropins the mean number of adult dark spermatogonia per tubular transverse section was 0.063. Of the 20 boys with normal mean adult dark spermatogonia per tubular transverse section 12 (60%) had good fertility potential, including a normal mean number of tubular germ cells, normal gonadotropins and normal inhibin B, compared to only 18 of 69 (26%) with an impaired mean number of adult dark spermatogonia per tubular transverse section ($p < 0.05$). Of 46 boys with a normal mean number of tubular germ cells 26 (57%) had placental-like alkaline phosphatase positive cells compared to 14 of 43 (33%) with a decreased mean number of tubular germ cells ($p < 0.05$).

Conclusions: The number of placental-like alkaline phosphatase positive gonocytes and adult dark spermatogonia per tubular transverse section are important parameters related to the fertility potential of boys with cryptorchid testes.

Key Words: testis; cryptorchidism; infertility, male; inhibin B; gonadotropins

The number of S/T in testicular biopsies is the parameter most often used to predict later fertility in adulthood in boys with cryptorchidism. Recently, by combining the results of serum gonadotropin and serum inhibin B blood samples, and determining the S/T in boys with bilateral

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cryptorchidism it was possible to identify those with a good prognosis at low risk for infertility, those with possible congenital abnormalities related to testicular descent at intermediate risk for infertility and those with insufficient gonadotropin stimulation as the primary pathogenic factor of cryptorchidism at related high risk for infertility.[1]

At birth patients with cryptorchidism harbor germ cells in the testes but in decreased number as a group compared to the normal population. From about age 15 months germ cells may be lacking.[2] The greater the age at surgery for cryptorchidism, the higher the risk of finding no germ cells in testicular biopsy at surgery, which predicts infertility in adulthood.[2,3] Probably induced by minipuberty, the neonatal gonocyte transforms into a type A spermatogonium during the first 12 months of age, a step that is now postulated to be crucial for subsequent fertility since stem cells for spermatogenesis are created in this structure.[3,4] Impaired transformation of neonatal gonocytes into type A spermatogonia during the first 12 months of life age with subsequent germ cell apoptosis may be a pathogenic factor for infertility.

Several groups identified a number of antigens that are highly expressed in the cells of intratubular germ cell neoplasia and gonocytes but not in normal germ cells of adult testes.[5] PLAP is a tissue specific alkaline phosphatase with unknown biological function that is expressed in classical seminoma, primordial germ cells, early neonatal gonocytes and fetal spermatogonia.[5,6] PLAP is one of the most commonly used markers for intratubular germ cell neoplasia and cancer in testicular biopsies.[5] However, in the testes of normal boys PLAP positive germ cells are present within year 1 of life.[6] PLAP positive gonocytes in normal testes within year 1 of life may indicate preserved ability for germ cell transformation and good fertility potential, as does the transformation of germ cells into a type Ad spermatogonium in cryptorchid testes within the first 12 months. However, it is uncertain how these parameters relate to total S/T and other factors associated with the fertility potential of boys with cryptorchidism, such as hormone profiles.

We hypothesized that the presence and number of PLAP positive germ cells and type A spermatogonia in undescended testes during early infancy would correlate with our mentioned recent classification of boys with bilateral cryptorchidism into 3 groups at low, intermediate and high risk for infertility.

MATERIALS AND METHODS

We prospectively included in the study 89 otherwise healthy, bilaterally cryptorchid boys 7 months to 3 years old (median age 1.8 years) who were seen consecutively by a single surgeon from 2008 to 2011. All had blood samples taken on the day of surgery and bilateral testicular biopsy was performed in all at orchiopexy. We excluded from analysis patients with chromosomal abnormalities, associated anomalies or previous inguinal surgery. None received hormonal therapy.

Patients were divided into groups depending on whether the S/T number was normal or decreased, whether serum FSH was normal or increased and whether preoperative serum inhibin B was normal or decreased. Criteria for classifying patients based on 75% of the current material were described previously.[1] In group 1 at intermediate risk for infertility all patients had preoperative serum FSH 1.3 IU/L or greater. In group 2 at high risk for infertility all patients had normal FSH, low S/T and/or low inhibin B. In group 3 at low risk for infertility all patients had normal S/T, normal FSH and normal inhibin B.

Hormonal Assays

Blood samples were obtained by venipuncture between 8:00 and 11:00 a.m. Serum samples were separated from the clot by 10-minute centrifugation at 2,000 × gravity. Serum was stored at −80C until analysis. Serum inhibin B was measured using an inhibin B enzyme-linked immunosorbent assay kit (Serotec, Oxford, United Kingdom) with a research kit, as recommended by the manufacturer. The lower detection limit was 5 pg/ml and measurements were made in duplicate. A similar inhibin B kit was predominantly used in previous studies of inhibin B in normal boys and those with cryptorchidism.[7] Normal serum inhibin B reference levels were defined as described by Andersson et al (fig. 1).[8]

LH and FSH were measured by sandwich electrochemiluminescence immunoassay. The lowest measured

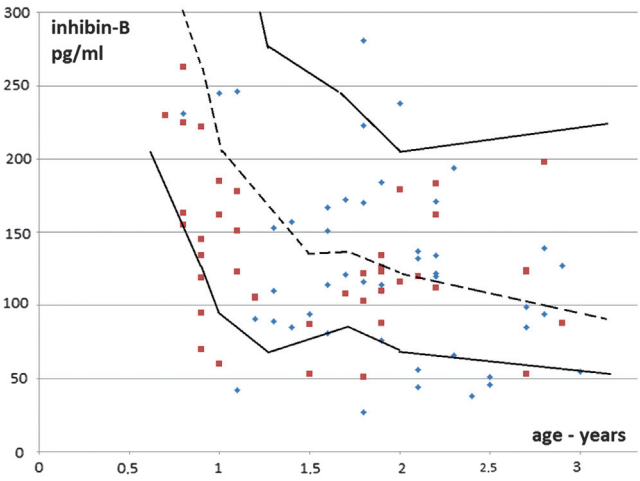

Figure 1. Serum inhibin B by age at surgery in patients with cryptorchidism and PLAP positive (red squares) vs negative (blue diamonds) germ cells in testicular biopsies. Normal reference serum inhibin B levels were considered those described by Andersson et al.[8] Solid curves indicate lower and upper normal ranges. Dashed curve indicates median.