Age at Orchiopexy and Testis Palpability Predict Germ and Leydig Cell Loss: Clinical Predictors of Adverse Histological Features of Cryptorchidism

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Abbreviations and Acronyms

UCSF = University of California–San Francisco

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Purpose: We determined the relationship between clinical variables and testicular histopathological changes associated with decreased fertility potential in children with cryptorchidism.

Materials and Methods: Testis biopsies of 274 children who underwent orchiopexy and concurrent testicular biopsy between 1991 and 2001 were analyzed for germ and Leydig cell loss, and testicular fibrosis. Multivariable logistic regression was used to determine if age at orchiopexy (analyzed as continuous and ordinal variables), preoperative testis palpability, unilateral vs bilateral disease and/or side of undescended testis was predictive of these pathological outcomes.

Results: Age at orchiopexy was associated with germ and Leydig cell depletion. Each month of testis undescent was associated with development of moderate/severe germ cell depletion (OR 1.02 for each month of age, $p < 0.005$) and Leydig cell loss (OR 1.01 for each month of age, $p < 0.02$). Nonpalpable testes were associated with severe germ cell depletion. Children with palpable testes had lower odds of germ cell depletion than those with nonpalpable testes (OR 0.46, $p < 0.005$). This finding corresponds to a significant 2% risk per month of severe germ cell loss and 1% risk per month of Leydig cell depletion for each month a testis remains undescended, and a 50% greater risk of germ cell depletion in nonpalpable relative to palpable cryptorchid testes.

Conclusions: Testes that remain undescended are associated with progressive loss of germ and Leydig cells, and nonpalpable testes predict severe germ cell loss.

Key Words: cryptorchidism, fertility, testis, urogenital surgical procedures

Cryptorchidism is a common developmental abnormality with a prevalence of 1% to 3% in full-term and 15% to 30% in premature male infants. Approximately 20% of patients with cryptorchidism have bilaterally undescended testes.[1] Cryptorchidism is associated with impaired fertility.[2] Patients with bilateral undescended testes have a lower paternity rate than those with unilateral cryptorchidism or normally descended testes, although unilateral cryptorchidism is associated with variable rates of infertility.[3,4]

Previous studies have suggested that orchiopexy be performed before age 12 months to minimize germ cell loss since it has been shown that fertility index (defined as number of spermatogonia per tubule) is adversely affected in children with

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cryptorchid testes after age 1 year. It is hypothesized that the longer duration of testis undescent correlates with higher rates of germ cell loss and adult infertility.[5–7] The specific etiology for this compromised infertility remains unclear but it is likely related to germ cell depletion and/or defective germ cell maturation, loss of Leydig cells and/or an increase in testicular fibrosis.[8,9] These histological changes are associated with abnormal semen parameters in these patients during adulthood.[10,11] If cryptorchidism is a progressive disease, as postulated by Hadziselimovic et al,[12] then the number of germ cells and/or Leydig cells should correspondingly be lower in patients whose testes remain undescended for longer periods.

The decision to refer to a pediatric urologist is based on the unlikelihood of spontaneous testis descent with increasing age, as well as the desire to minimize compromised fertility potential. The literature generally supports orchiopexy before age 1 year because of surgical safety considerations, and because histological and clinical fertility parameters are demonstrably improved in children who undergo orchiopexy before age 2 years.[13–16] Additionally recent studies in preclinical models suggest that early orchiopexy can halt testicular degeneration caused by undescent.[17]

We determined if a defined set of readily available clinical factors (age at orchiopexy, preoperative ability to palpate the testis, unilaterality vs bilaterality and side of undescended testis) were associated with the well-defined histological changes seen in cryptorchid testes using standard paraffin preparation and light microscopic analysis of testis biopsies. This method reportedly is as reliable in determining fertility index as semithin (0.5 micron) sections, which are more costly and difficult to prepare.[5] We hypothesized that testicular fibrosis, and loss of germ and Leydig cells more likely would occur in children who underwent orchiopexy later in life and/or had nonpalpable or bilateral undescended testes. To our knowledge this is the first study to identify specific clinical variables associated with progressive pathological changes in cryptorchid testes. In doing so we also attempted to determine the optimal timing for orchiopexy to minimize future infertility associated with these pathological changes.

MATERIALS AND METHODS

Study Design

The study was approved by the UCSF Committee on Human Research. We reviewed the charts of all patients younger than 18 years who underwent orchiopexy at UCSF between 1991 and 2001. At that time orchiopexy with concurrent testicular biopsy was considered the standard of care, and biopsy was performed in the undescended testis of all children whose parents gave informed consent. Only patients who underwent testicular biopsy at the time of orchiopexy were included in the study. Patients who underwent orchiectomy for nonviable testes were excluded. A total of 318 children met inclusion criteria. Of these patients only individuals with complete pathological assessment of germ cell depletion, presence or absence of Leydig cells and degree of interstitial fibrosis were included (290). Clinical data were missing in 16 patients, thus allowing 274 to be included in the final analysis.

Any pathology report with ambiguous conclusions prompted re-review of primary pathological specimens. The slides were read by multiple surgical pathologists at UCSF using a standardized synoptic that was introduced in 1997. Leydig cells were reported as absent or present; fibrosis as absent, mild, moderate or severe; and number of germ cells as average number per tubule. The slides were re-read by a single pathologist (GEK) in cases that were originally read before introduction of the synoptic.

Pathology

Three to 4 micron hematoxylin and eosin stained sections of archival Bouin fixed, paraffin embedded tissue were evaluated for the histological features of 1) degree of peritubular fibrosis, 2) average number of germ cells per tubule and 3) presence or absence of Leydig cells (see figure). For this study the entire specimen was reviewed. Peritubular fibrosis, which was determined by the average appearance of the specimen, was graded as none, mild, moderate or severe. The area of the biopsy that contained the most germ cells at low power was used to establish average number of germ cells per tubule. These areas were representative of the entire specimen, as only 1 biopsy had focal spermatogenesis. Leydig cells and/or their mesenchymal precursor cells, which are present before age 7 years and can be seen under light microscopy, were classified as absent or present after review of the entire specimen.[18]

Statistical Analysis

Pathology data were reported as dichotomous variables (germ cell depletion as absent/mild or moderate/severe, Leydig cells as present or absent and fibrosis as absent/mild or moderate/severe). Moderate/severe germ cell depletion was defined as less than 67% of tubules containing at least 1 germ cell. Degree of germ cell depletion was based on thirds, with mild depletion defined as greater than 66% of tubules containing at least 1 germ cell, moderate depletion as 33% to 66% of tubules containing at least 1 germ cell and severe depletion as less than 33% of tubules containing at least 1 germ cell. Due to the relative scarcity of Leydig cells and their precursors, a graded assessment of depletion could not be performed and, thus, they were reported as present or absent. All predictor values were also dichotomous except for age, which was analyzed as a continuous variable and an ordinal variable in 4 categories consisting of 0 to 12 months, 13 to 24 months, 25 to 96 months and greater than 96 months.

Data were analyzed using Stata® software. Multivariable logistic regression was used with 3 different outcomes—presence or absence of moderate or severe germ