ANATOMICAL STUDIES OF THE FETAL GENITALIA: SURGICAL RECONSTRUCTIVE IMPLICATIONS

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INTRODUCTION

Under the influence of the SRY gene, testes determining factor, and androgens fetal differentiation precedes to form the normal male genitalia. Genetic and environmental abnormalities can disrupt normal development leading to an ambiguous state of the genitalia. In most societies it is the accepted norm; that the genitalia either look male, or female, with an ambiguous state not being socially acceptable. Based on societal norms, the previous standard of care was to surgically recreate either the male or the female physical appearance. Long-term data validating this approach, however, is lacking and recently there has been an effort to document the optimal treatment for patients who do not fit into either a classic male or female category. The new concept that is gradually being embraced is that sex assignment is not an irrevocable phenomena. In other words, it is not a social or medical emergency to assign a sex immediately after birth, and in fact, the sex of the patient may actually change during life depending on genotypic, phenotypic and environmental conditions. This awareness has been initiated by both physicians as well as advocate groups with the hope that difficult but correct decisions can be made for patients with ambiguous states in the future.

In an attempt to be able to understand the anatomy of both normal and abnormal genital development, we have embarked on an extensive histologic analysis of fetal specimens. These studies by their inherent nature are not complete but are in progress and depend on the availability of adequate tissue for analysis. The present paper is based on over 50 fetal specimen that have been carefully analyzed. The anatomy of the human genitalia has been looked at from a structural, cellular, neural and vascular point of view. The implications of the fetal anatomy has been applied to the strategic design of reconstructive procedures.

Materials and Methods

These investigations have been approved by the Institution’s Committee on Human Research. Human fetal male and female specimens between 8weeks and the newborn period were analyzed after being embedded in paraffin and serial sectioned (6µ). Specimens with hypospadias were also studied. Fetal sex was determined by fluorescence in situ of the X and Y chromosome specific probes. The entire fetal specimen was preserved with each slide containing 2 to 4 histologic sections. Specimens

Pediatric Gender Assignment: A Critical Reappraisal
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were dated based on fetal femur length when possible, or fetal heal-toe length. Staining was performed with hematoxylin and eosin and Masson's trichrome. Immunohistochemical staining was performed selectively with antibodies raised against smooth muscle α-actin, neural markers S100 and PGP 9.5, epithelial cyto-keratin markers and markers for blood vessels, such as von Willebrand's and factor 8. The avidin-biotin peroxidase procedure was used with Vectastain ABC kits (Vector Laboratories, Burlingame, CA) and cobalt intensification. Antibodies against smooth muscle α-actin and the neuronal markers S100, as well as the cytokeratins were obtained from Sigma (St. Louis, MI). Anti-neuronal cytoplasm protein gene product 9.5 was obtained from ultra clone in the island Well's United Kingdom. Double immunostaining for both smooth muscle, α-actin and the neuronal markers S100 and PGP 9.5 were performed using biotinylated secondary antibody obtained, obtaining a brown color with antimouse-IgG from sheep. A pink stain was obtained using biotinylated secondary antibody antimouse IgG made from horse and labeled with alkaline phosphatase factor AK-5002. The substrate was Vector Red (Vector SK-5100) (Vector Laboratories Burlingame, CA).

Anatomical three-dimensional computer reconstruction was created of normal male, female and specimens with hypospadias using an Olympus digital camera, Adobe Photoshop, 5.1, NIH imaging and an Apple computer, as described in previous publications (Baskin et al, 1997, 1998, 1999). In short, every 10th to 20th section was imaged and then digitized. The corporal or erectile body, glans, foreskin, clitoral hood and areas of interest were manually outlined. Nerves and vascular structures as well as the corporal erectile tissue outlined were contrasted and enhanced with the respective background. Accuracy of the enhanced images was checked against still photographs of the original immunostained specimens. Three-dimensional reconstruction was performed in the X and Y axis. Animated motion picture and views of interest were captured as images. Coloring of the genital anatomical structures was artificially assigned.

RESULTS

Normal Human Fetal Penis

Localization of nerves with anti-PGP neuronal marker was noted in specimens of all fetal ages—prominent dorsally at 11 and 1 o'clock, but also extending around the tunica to the junction of the corpus spongiosum and corpora cavernosa suggesting that we may be injuring these structures in penile straightening procedures (Baskin et al, 1996, 1997). (Figure 1 & 2) The nerves continued into the glans on the dorsal aspect suggesting that glans reduction in feminizing genitoplasties should be performed on the ventral aspect.

Smooth muscle was first noted at 10 weeks' gestation, with epithelial differentiation occurring in the earliest specimens studied (8 weeks' gestation) (Baskin et al, 1997). Over time, smooth muscle density was highest in the corpus spongiosum, especially between urethra and the corpora cavernosa. Smooth muscle also developed in close proximity to the urethral epithelium. The tunica albuginea showed consistent variations