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Morphology of the fetal bladder during the second trimester: Comparing genders

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Abstract *Objectives:* The aim of the present study was to determine, by histological and stereological analysis, whether there are between-gender structural differences in the bladder in the second gestational trimester in human fetuses.
Material and methods: Forty bladders, which were obtained from 40 human fetuses (20 males and 20 females) ranging in age from 13 to 23 weeks post-conception (WPC), were studied. The fetuses were macroscopically well preserved, without anomalies of the urinary and genital systems; the cases with syndromes were abandoned. The bladders were dissected and embedded in paraffin, from which 5-μm thick sections were obtained and stained with: Masson's trichrome, to quantify connective and smooth muscle tissue; Weigert's resorcin fuchsin, to observe elastic fibers; picrosirius red with polarization, to observe collagen; and anti-beta III tubulin antibody, to observe the bladder nerves. The images were captured with an Olympus BX51 microscope and Olympus DP70 camera. The stereological analysis was performed with the Image Pro and Image J programs, using a grid to determine volumetric densities (Vv). Means were statistically compared using simple linear regression and the paired *t*-test ($P < 0.05$).
Results: The fetuses weighed between 60 and 490 g, and had crown—rump lengths between 9.5 and 20.4 cm. No elastic system fibers were observed in any bladders. Quantitative analysis indicated no differences in the Vv of the smooth muscle cells in the male bladders (26.19—50.16%; mean = 35.66%) compared to the female ones (30.60—45.63%; mean = 38.73%) ($P = 0.740$) and there were also no differences in the Vv of the connective tissue in females (40.52—60.40%; mean = 50.69%) and males (38.84—70.16%; mean = 57.04%) ($P = 0.0506$). There were no differences observed in the distribution of the nerves and collagen between the genders.

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Conclusion: The histological analysis of the smooth muscle, collagen, nerves and connective tissue of the developing bladders revealed that there are no gender differences during weeks 13—23 of gestation.

Introduction

The fetal bladder can be identified in the tenth week post-conception due to the beginning of urine production [1]. The bladder is formed from mesenchyme and endodermal cells [1].

Some bladder pathologies have different behaviors between genders, especially primary VUR, which is generally more severe in male fetuses and associated with thickening of the bladder wall [2]. The second trimester is very important in bladder embryonic development [1]. The development of the prostate during the second gestational trimester, which is associated with the production of hormones by the fetal testes, is a factor that can explain transient urethral obstruction in male human fetuses [3,4].

Studies of development of the bladder, vesical trigone and bladder neck are frequent in the literature [5—8]; however, specific studies of morphological differences in the fetal bladder musculature between genders are rare [9].

The aim of the present study was to determine, by histological and stereological analysis, whether there are between-gender structural differences in the bladder in the second gestational trimester in human fetuses.

Material and methods

The present study was carried out in accordance with the ethical standards of the hospital's institutional committee on human experimentation. Forty bladders, obtained from 40 human fetuses (20 males and 20 females) ranging in age from 13 to 23 weeks post-conception (WPC) during the period from January 1996 to April 2014, were studied. The maternity department of the hospital, with parent approval, obtained all of the fetuses. The fetuses were macroscopically well preserved, without anomalies of the urinary and genital system; the cases with syndromes were abandoned.

The gestational ages of the fetuses were determined in WPC according to the foot-length criterion, which is currently considered to be the most acceptable parameter to calculate gestational age [10—12]. Immediately before dissection, the fetuses were also evaluated regarding crown—rump length (CRL) and body weight. The same observer analyzed the measurements.

After the measurements were taken, the fetuses were carefully dissected with the aid of a stereoscopic lens with 16/25× magnification. The abdomen and pelvis were opened to identify and expose the urogenital organs. The bladder was separated from the other structures, and sections of the dome were fixed in 10% buffered formalin, and routinely processed for paraffin embedding, after which 5-μm thick sections were obtained at 200-μm intervals.

Smooth muscle, connective tissue, elastic system fibers, nerves and collagen were studied by histochemical and immunohistochemical methods. The sections were stained with hematoxylin—eosin to assess the integrity of the tissues. The following stainings were also performed: Masson's trichrome, to quantify connective tissue and smooth muscle; Weigert's resorcin fuchsin with previous oxidation, to observe elastic system fibers; picrosirius red with polarization, to observe different collagen types; and tubulin (anti-beta III mouse monoclonal antibody) (http://www.pierce-antibodies.com/beta-3-Tubulin-antibody-clone-TU-20-Monoclonal-MA119187.html), to observe the bladder nerves. Connective tissue, smooth muscle and elastic system fibers were quantified by a stereological method [13] Table 1.

Five sections were stained and five fields of each section were selected. All selected fields were photographed with a digital camera (DP70, Olympus America, Inc., Melville, New York) under the same conditions at a resolution of 2040 × 1536 pixels, directly coupled to the microscope (BX51, Olympus America, Inc.) and stored in a TIFF file. The Image J software, version 1.46r, loaded with its own plug-in (http://rsb.info.nih.gov/ij/) was used to determine the volumetric density (Vv) of each component. Results for each field were obtained through the quantification assessment method, by superposing a 100 points test grid (multipurpose test system) on the video monitor screen (Fig. 1). The arithmetic mean of the quantification in five fields of each section was determined. Afterwards, the mean quantification value for the five sections studied from each bladder (total of 25 test areas) was obtained.

The data were analyzed by simple linear regression (to obtain the coefficient r for each regression analysis) and the paired t-test to assess the association between the variables with fetal age and other variables, with the GraphPad Prism 5.0 software. All tests were two-sided and a P-value <0.05 was considered to be statistically significant.

Results

The fetuses ranged in age from 13 to 23 WPC, weighed between 60 and 490 g, and had crown—rump lengths between 9.5 and 20.4 cm. No elastic system fibers were observed in any bladders (Fig. 2). This may indicate that in fetal bladders, this extracellular matrix component appears only in the third gestational trimester. Quantitative analysis indicated no differences in the Vv of the smooth muscle cells in the male bladders (26.19—50.16%; mean = 35.66%)