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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%)

compared to the female ones (30.60–45.63%; mean = 38.73%) (P = 0.740) and no differences were detected in the Vv of the connective tissue in females (40.52–60.40%; mean = 50.69%) compared to males (38.84–70.16%; mean = 57.04%) (P = 0.0506).

When comparing the gestational age with smooth muscle, no correlation was found between female (r^2 = 0.3986) or male fetuses (r^2 = 0.02661). When comparing the gestational age with connective tissue, no correlation was found between female (r^2 = 0.6282) or male fetuses (r^2 = 0.03060); Fig. 3 shows the correlations graphs. Although there were no correlations between the means, according to gender and age, the curves show a tendency for reduction of the connective tissue with age and an increase in the muscle tissue with age in the female fetuses, but not in the males.

No differences were observed in the distribution of the nerves in the bladder wall between the male and female fetuses. There were no significant differences noted in the qualitative analysis of the bladder collagen between genders.

Table 1 Age and fetal parameters of study samples, and the measurements of the muscle and connective tissue in male and female fetuses.

Age (WPC)/Fetal gender	Weight (g)	CRL (cm)	Connective tissue (%)	Muscle (%)
13.4/F	60	9.5	58.64	30.60
13.7/F	120	12.2	57.90	30.66
14.5/F	105	12.5	59.71	32.85
15.6/F	190	13.0	58.16	36.24
15.7/F	190	14.0	57.63	37.38
16.1/F	200	16.0	48.67	40.97
16.5/F	220	16.0	46.91	45.63
16.5/F	220	15.9	60.40	45.04
16.6/F	225	16.0	47.32	34.72
17.4/F	280	16.0	50.66	39.14
17.4/F	290	16.0	52.48	40.07
17.8/F	280	15.5	50.60	37.16
18.0/F	300	16.5	41.48	42.08
18.2/F	405	18.0	44.75	40.22
18.4/F	350	17.0	47.56	40.32
18.6/F	335	16.5	50.32	40.76
19.0/F	300	18.9	47.34	39.11
19.4/F	400	18.0	46.10	41.82
19.5/F	285	18.5	46.80	40.48
20.4/F	455	19.3	40.52	39.40
13.4/M	90	12.0	57.14	31.12
14.3/M	120	12.3	58.72	33.64
14.7/M	165	13.0	57.79	34.76
15.4/M	125	13.3	60.64	33.76
15.5/M	190	13.0	60.22	32.38
15.9/M	185	14.5	60.98	32.56
16.4/M	245	16.5	68.12	26.69
16.6/M	150	14.5	68.15	26.19
16.6/M	185	15.0	69.96	27.08
17.3/M	280	17.0	48.64	42.72
17.3/M	300	16.9	48.04	42.76
17.4/M	245	15.0	48.48	45.88
17.6/M	290	16.0	49.13	43.45
18.0/M	280	16.0	52.12	39.98
18.0/M	350	16.8	62.20	30.76
18.0/M	350	17.7	53.84	41.16
18.0/M	365	18.5	38.84	50.16
20.1/M	380	18.8	61.96	32.68
21.4/M	455	19.3	70.16	27.00
23.0/M	490	20.4	45.70	38.52
Mean	**261.25**	**15.79**	**53.86**	**37.19**

M = Male; F = Female; WPC = weeks post-conception; g = grams; CRL = crown–rump length; cm = centimeters.

Discussion

Most of the urinary bladder originates from the vesical part of urogenital sinus, while the trigone results from the absorption of the caudal region of the mesonephric duct during development of the bladder [1,14]; however, some recent studies, using various methods including cell lineage, have shown that the trigone is derived from the endoderm, not from the mesoderm [15,16]. During the second gestational trimester, the bladder presents a series of developmental changes, finally acquiring the urothelial lining and a well-developed muscular coat [1,14,17].

Freedman [18] studied the bladder structure of nine fetuses with PUV and urethral atresia. That study demonstrated a substantial increase in the thickness of the bladder wall, but no significant alterations were observed in the distribution of the connective tissue and smooth muscles of the fetal bladder. It also suggested that the intra-utero obstruction processes are not associated with excess collagen deposition, but rather with an increase in the development of the vesical musculature [18].

The growth of the prostate during the second gestational trimester, leading to the occurrence of a transient infravesical obstruction, was noted in a previous study [3]. The increase in the prostate volume in the fetal period appears to be a determining factor for significant differences in the structure of the bladder neck and internal urethral

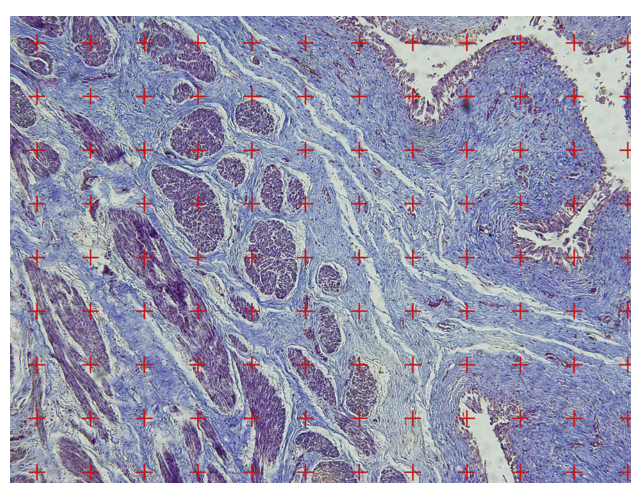

Figure 1 Morphometric analysis of the fetal bladder. The photomicrograph shows the quantification of smooth muscle cells of a 16-week post-conception male fetus bladder using the Image J Test grid software. Masson's trichrome ×400.

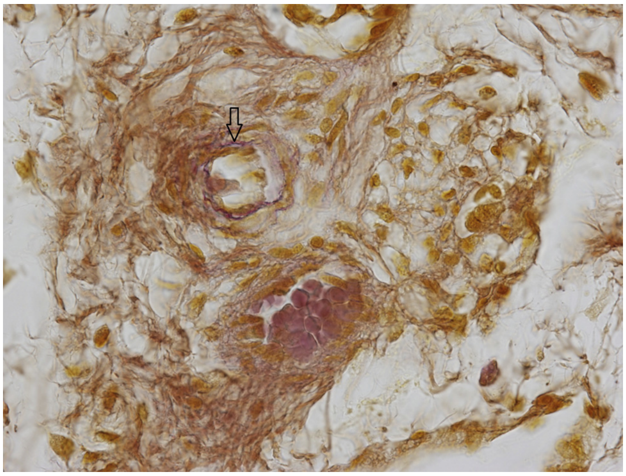

Figure 2 Elastic system fibers. The photomicrograph shows a 21-week post-conception female fetus bladder. Elastic fibers can only be observed in the wall of an artery (arrowhead). Weigert ×1000.

sphincter in male fetuses [7]. Nevertheless, to date, no study has demonstrated structural differences in the bladder wall between genders in the human fetal period.

In a study with 48 human fetuses aged between 9 and 35 WPC, Koerner [9] analyzed the bladder musculature by immunohistochemical techniques and suggested that in normal fetal growth, detrusor muscle formation proceeds independently of genital sex. The present study included a significant number of male and female fetuses from the second gestational trimester. Besides the musculature, the connective tissue, collagen and elastic fibers of the fetal bladder were analyzed and no significant differences between genders were observed in the distribution of these components of the bladder wall. When the gestational age was compared with smooth muscle and connective tissue, no correlation was found in male or female fetuses.

Qualitative immunohistochemical techniques were also applied to analyze the distribution of the bladder nerves of the male and female fetuses, and no significant differences were found between genders. In a previous study only investigating male fetuses, the authors demonstrated the presence of paraganglion cells within the muscle coat of the fetal bladder [19]. In another study with eight adult cadavers and five fetuses aged between 20 and 30 WPC, for the purpose of avoiding injuries to nerves during pelvic surgery, the authors observed the places where the nerves enter the bladder [20]. The present study contains the first published comparative analysis of the bladder nerves of male and female fetuses in the second gestational trimester.

Pazos [21] studied the bladders of anencephalic fetuses and demonstrated a greater amount of connective tissue than in the normal fetuses' bladders. This suggests an alteration in the complete development of the smooth

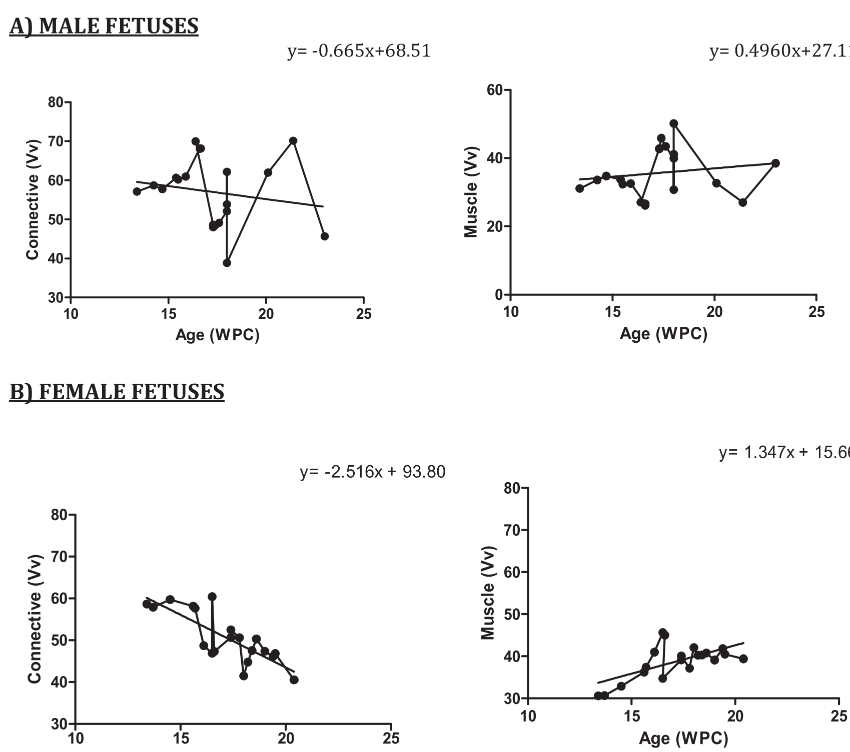

Figure 3 The correlation between smooth muscle and connective tissue with age in male and female fetuses. Points plotted represent values obtained for each week studied. A) Correlation of smooth muscle and connective tissue in male fetuses. Linear regression indicates that when the gestational age was compared with smooth muscle ($r^2 = 0.02661$) and connective tissue ($r^2 = 0.03060$) no correlation was found in male fetuses. B) Correlation of smooth muscle and connective tissue in female fetuses. Linear regression indicates that when the gestational age was compared with smooth muscle ($r^2 = 0.3986$) and connective tissue ($r^2 = 0.6282$) no correlation was found in female fetuses.

muscle layer. The lesion in the nervous system, with consequent alteration in bladder nerve regulation, could be a plausible hypothesis to explain these structural changes. Bladder nerves in anencephalic fetuses could be modified due to cerebral lesions, with consequent brain control damage in bladder nerves; however, in that study, the distribution of bladder nerves in anencephalic fetuses was not investigated, so further research is required to confirm or refute this hypothesis.

Collagen and elastin are important components of the bladder wall as they affect bladder function. Collagen provides tensile strength, but over-accumulation can inhibit bladder contractility and the conduction of electrical impulses through the wall [22]. Smooth muscles, having no tendons, require more collagen. In the present study, the qualitative analysis did not reveal differences the distribution of collagen in the fetal bladder wall between the genders. Elastin provides tissue elasticity and should help tissue compliance [23]. Elastic system fiber alterations are involved in fibrotic tissue formation; however, in the samples in the present study, no elastic fibers were seen in any of the bladders. This may indicate that in fetal bladders, this extracellular matrix component appears only in the third gestational trimester. Previous studies have shown the existence of elastic system fibers in other human fetal genitourinary organs [24].

In the present study, no gender differences were found in the bladder walls of second-trimester fetuses, which is the most important period for growth and development of the bladder.

Conclusions

The histological analysis of the developing bladder reveals that the smooth muscle, collagen, nerves and connective tissue are similar between genders during weeks 13–23 of gestation.

Conflict of interest

No conflicts.

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