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Morphology of the fetal renal pelvis during the second trimester: Comparing genders☆

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ABSTRACT

Objectives: Many studies of neonates have shown that renal pelvis ectasia is more common in boys. The aim of this study was to determine whether there are structural differences in the renal pelvis between male and female fetuses in the second trimester of gestation.

Material and methods: We studied 34 renal pelvises obtained from 34 human fetuses (17 males and 17 females), ranging in age from 13 to 23 weeks postconception. The renal pelvis tissue was stained with Masson's trichrome to quantify connective and smooth muscle cells (SMC). The tissue also was fixed for scanning electron microscopy (SEM) in a modified Karnovsky solution. The images were captured with an Olympus BX51 microscope and Olympus DP70 camera. The stereological analysis was done with the Image-Pro and ImageJ programs, using a grid to determine volumetric densities (Vv). Means were statistically compared using simple linear correlation and the Mann-Whitney test ($p<0.05$).

Results: Quantitative analysis indicated differences ($p=0.0275$) in Vv of connective tissue in male renal pelvises (mean = 55.3%) compared to female ones (mean = 51.46%). Quantitative analysis indicated a significant difference ($p=0.0002$) in SMC in male renal pelvises (mean = 12.57%) compared to female ones (mean = 16.22%). When we compared the SMC at different ages, we did not find any correlation in male ($r^2=0.2657$, $p=0.3027$) or female fetuses ($r^2=0.3798$, $p=0.1326$). When we compared the connective tissue at different ages, we did not find any correlation in female fetuses ($r^2=0.3798$, $p=0.2870$), but we did observe a positive correlation between the connective tissue and age in male fetuses ($r^2=0.8308$, $p<0.0001$). SEM showed that the collagen fibers had no differences between male and female.

Conclusion: The renal pelvis presents significant structural differences between male and female fetuses. The renal pelvis in males had less SMC and presented a positive correlation of connective tissue with age and the renal pelvis in female had less connective tissue without correlation with the age.

Level of evidence: III

The second gestational trimester is very important for the embryonic development of the kidneys, renal pelvis, bladder and ureter [1,2]. An important branching of the ureteric bud occurs between the 5th and 14th weeks postconception, leading to formation of the major and minor renal calyces, renal pelvis and collecting tubules [3,4]. The process of kidney development is completed by the 34th week of gestation in humans [5].

Many studies, both in fetuses using prenatal ultrasound and in neonates, have shown that renal pelvis ectasia is more common in boys [6,7]. Some urinary pathologies have different behavior between the sexes, especially primary vesicoureteral reflux (VUR), which is generally more severe and combined with kidney damage in male fetuses [8]. We can speculate that the cause of the changes in urinary system is increased pressure from the smaller urethral caliber in males, but the bladder does not show a distinction in SMC and connective tissue between the sexes [9].

Studies of development of the kidney, ureter and ureteral bud branching are frequent in the literature [2,4,6]. Previous studies about the histology of ureteropelvic junction obstruction helps in understanding the etiology of this congenital anomaly [10,11]. However, specific studies of morphological differences in the fetal renal pelvis structure comparing genders are absent in the literature. In the present paper we analyzed human fetuses and studied the fetal renal pelvis by histological and scanning electron microscopy techniques used in previous studies in human fetuses and pediatric patients [12–15].

The hypothesis of this paper is that the renal pelvis presents structural differences between male and female fetuses which

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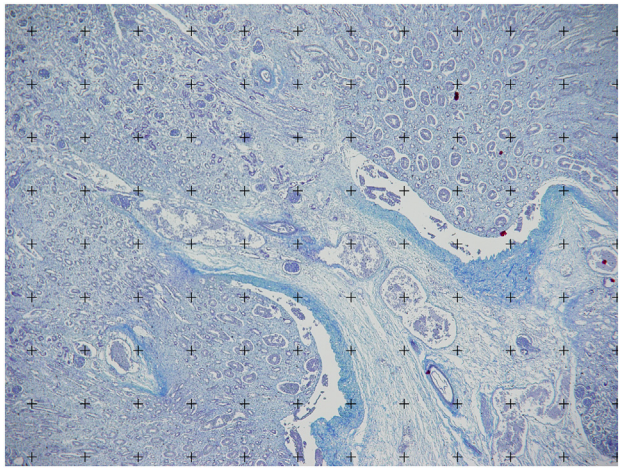

Fig. 1. Morphometric analysis of the fetal renal pelvis. The photomicrographs show the quantification of smooth muscle cells and connective tissue of the renal pelvis in a male fetus with 21 WPC using the Image J Test grid software. Masson's trichrome ×200.

may be a predisposing factor for the higher incidence of urinary pathologies in male fetuses. Therefore the aim of this study is to determine whether there are structural differences in the renal pelvis between human male and female fetuses in the second trimester of gestation.

1. Methods

The experimental protocol described here was approved by the Ethics Committee on Human Experimentation of our university (number: 2.079.618, CAAE: 67944217.3.0000.5259). This study was also carried out in accordance with the ethical standards of the hospital's institutional committee on human experimentation.

We studied 34 renal pelvises obtained from 34 human fetuses (17 males and 17 females), ranging in age from 12 to 23 weeks postconception (WPC), during the period from January 2017 through January 2019. The fetuses came to our laboratory as a donation of the Obstetric section of our hospital. The fetuses were macroscopically well preserved, showed no signs of malformations and the demise is hypoxia. The gestational age was determined in WPC according to the foot-length criterion. This criterion is currently considered the most acceptable parameter to estimate gestational age [16–19]. The fetuses were also evaluated regarding crown-rump length (CRL) and body weight immediately before dissection. The measurements were taken with the help of a magnifying lens and a digital pachymeter (calibrated in millimeters). The same observer made all the measurements. All the kidneys with anomalies (fusion, rotation, duplication) and renal pelvis dilation were excluded from the study.

After the measurements, the fetuses were carefully dissected with the aid of a stereoscopic lens with 16/25× magnification. The kidneys were removed together with the ureters, bladder and genital organs. The renal pelvises were separated from the other structures and fixed in 10% buffered formalin, and routinely processed for paraffin embedding, after which 5-μm thick sections were obtained at 200-μm intervals

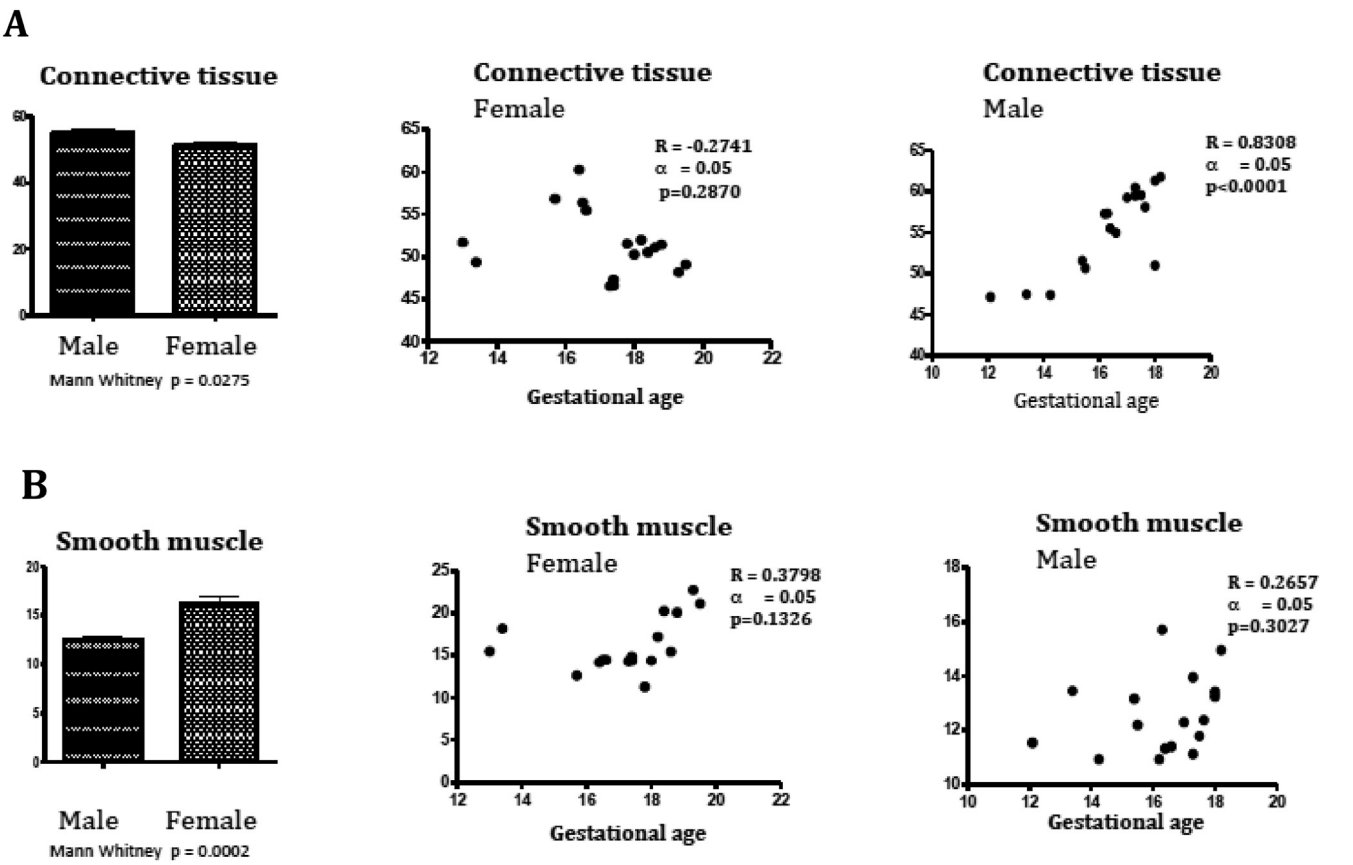

Fig. 2. Quantitative analysis of fetal renal pelvis. (A) Connective tissue. The Mann–Whitney test showed significant differences in volumetric density of the connective tissue between male and female fetuses ($p = 0.0275$). The linear regression indicated that connective tissue is correlated significantly and positively with fetal age in male fetuses ($r^2 = 0.8308$, $p < 0.0001$). In female fetuses, the connective was not significantly correlated with different ages ($r^2 = 0.3798$, $p = 0.2870$). (B) Smooth muscle cells (SMC). The Mann–Whitney test showed a significant differences in volumetric density of the SMC between male and female fetuses ($p = 0.0002$). Linear regression indicated no correlation in male ($r^2 = 0.2657$, $p = 0.3027$) or female fetuses ($r^2 = 0.3798$, $p = 01326$) in SMC at different ages.

and stained with Masson's trichrome to quantify connective and smooth muscle cells (SMC) and connective tissue, and with Weigert's resorcin fuchsin to observe elastic system fibers. Connective tissue, smooth muscle tissue and elastic system fibers were quantified by a stereological method [20–22].

We studied 5 microscopic fields chosen at random, totaling 25 test areas studied for each renal pelvis for the quantitative analysis. We used the Image J software, version 1.46r, loaded with its own plug-in (http://rsb.info.nih.gov/ij/). All sections were photographed with a digital camera (DP70, Olympus America, Inc., Melville, New York) under the same conditions at a resolution of 2.040 × 1.536 pixels, directly coupled to the microscope (BX51, Olympus America, Inc.) and stored in a TIFF file.

To quantify the smooth muscle tissue and connective tissue, we used the Image J software to determine the volumetric density (Vv) of each component (Fig. 1). Results for each field were obtained through the quantification assessment method, by superposing a 100-point test grid (multipurpose test system) on the video monitor screen. The arithmetic mean of the quantification in 5 fields of each section was determined. Afterwards, we obtained the mean quantification value for the 5 sections studied from each renal pelvis (total of 25 test areas).

Small fragments of the renal pelvis were used to investigate the ultrastructure. The samples were submitted to fixation for scanning electron microscopy (SEM) by immersing tissue fragments in a modified Karnovsky solution for 48 h at 4 °C. This fixative consisted of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M of sodium phosphate buffer, pH 7.4. To better visualize the three-dimensional organization of the vesicle stroma under SEM, tissue samples were submitted to an alkali treatment to solubilize and remove cells. The obtained acellular preparations were then processed for high-vacuum SEM, and observations were performed with a LEO 435 (Zeiss, Oberkochen, Germany) scanning electron microscope with an acceleration voltage of 15 to 20 kV. The team members involved in analysis of the scanning electron microscopy were blinded to whether the renal pelvis was male or female.

1.1. Statistical analysis

Means were statistically compared using simple linear correlation and the Mann–Whitney test (p-value < 0.05 was considered statistically significant). In addition, the correlation coefficient (r^2) and the p-value were obtained for each regression analysis. The correlation coefficient values <0.4 were considered to reflect very weak correlations and r^2 values >0.7 to reflect strong correlation. $p \leq 0.05$ was considered to indicate statistical significance. The GraphPad Prism 5.0 software was used.

2. Results

The fetuses presented gestational ages between 12 and 23 WPC. No statistical difference was observed between genders (female fetuses: $p = 0.1943$; $r^2 = 0.3099$; and male fetuses: $p = 0.0935$; $r^2 = 0.4609$). The fetuses weighed between 60 and 490 g, and had crown-rump length between 9.5 and 20.4 cm. We did not observe elastic system fibers in any renal pelvis analyzed. The quantitative analysis of smooth muscle cells and connective tissue of renal pelvis in male and female fetuses can be observed in Fig. 2.

Quantitative analysis indicated significant differences ($p = 0.0275$) in Vv of connective tissue in male renal pelvises (mean = 55.3%)

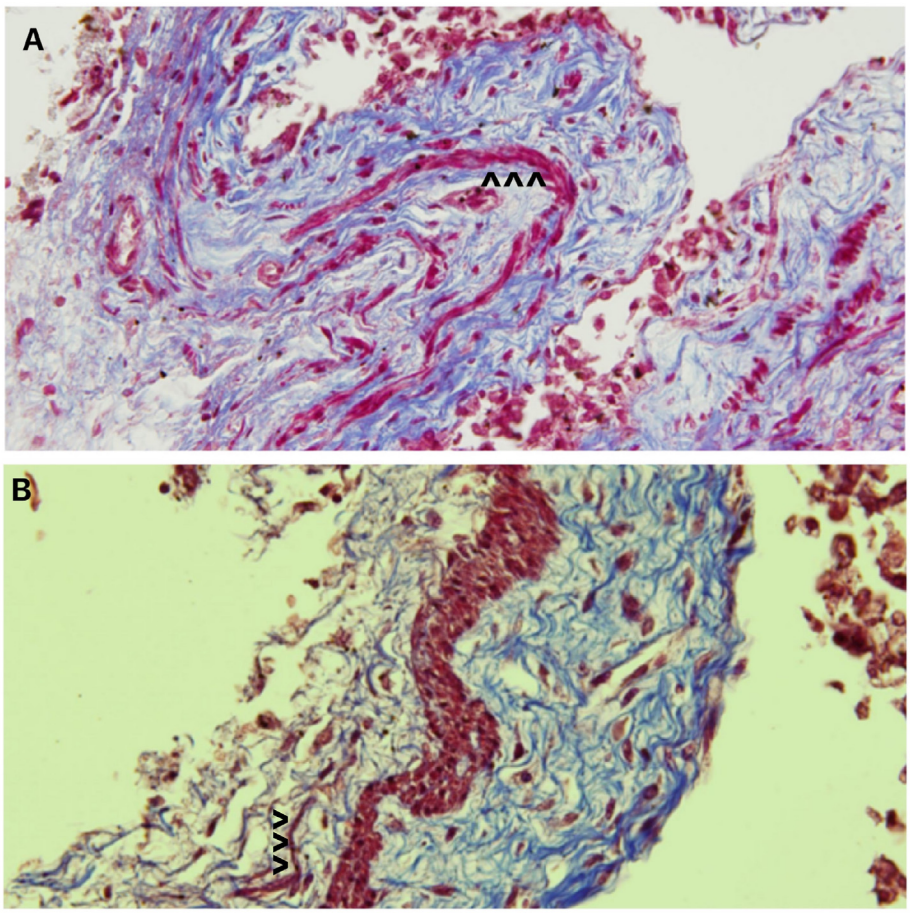

Fig. 3. Histology of Fetal Renal Pelvis. (A) Photomicrography of a renal pelvis in a female fetus with 16WPC. We can observe the smooth muscle marked with the arrowhead. Masson's trichrome ×200. (B) Photomicrography of a renal pelvis of a male fetus with 17WPC. We did not observe smooth muscle cells in this sample. We can observe the smooth muscle marked with the arrowhead. Masson's trichrome ×200.

compared to female ones (mean = 51.46%). Quantitative analysis indicated a significant difference ($p = 0.0002$) in smooth muscle cells in male renal pelvises (mean = 12.57%) compared to female ones (mean = 16.22%). When we compare the smooth muscle cells of fetal renal pelvises at different ages, we did not find any correlation in male ($r^2 = 0.2657$, $p = 0.3027$) or female fetus ($r^2 = 0.3798$, $p = 0.1326$). When we compare the connective tissue of fetal renal pelvis at different ages we did not find any correlation in female fetuses ($r^2 = 0.3798$, $p = 0.2870$), but we did observe a positive correlation between the connective tissue and age in male fetuses ($r^2 = 0.8308$, $p < 0.0001$). In Fig. 3 we can observe the representative histologic images that compare and show differences of male and female renal pelvis smooth muscle contents (Fig. 3).

We analyzed the alterations on the renal pelvis collagen of the male and female fetuses by scanning electron microscopy, with magnification of 5000×. SEM showed that the collagen fibers are made of thick fibrils in a tight parallel arrangement without differences between male and female. Fig. 4 depicts the analysis of collagen system distribution in fetal renal pelvis. It can be seen that the collagen fibers are densely packed in parallel undulating arrays and have the same organization in male and female fetuses.

3. Discussion

During the fourth or fifth WPC, the ureteric bud arises from the caudal segment of the Wolffian duct, grows cranially, and the interaction with the metanephric blastema is crucial to proper kidney formation [2]. The ureter, renal pelvis, calyces and collecting tubules develop from the ureteric bud. The metanephrogenic blastema forms glomeruli, proximal tubules and distal tubules. At nine weeks of development, the metanephros, which will become the mature kidney, starts to produce urine [2]. The renal pelvis is elastic and can alter its diameter according to the instantaneous volume of urine being produced, which changes according to physiological conditions [23].

Ureteropelvic junction (UPJ) obstruction is the most common cause of congenital hydronephrosis, and impaired collagen production by anomalous smooth muscle cells may be implicated in the origin of this anomaly [24]. The increase of collagen fibers as a result of dysfunction and atrophy of SMC can disrupt the mobility of the UPJ and lead to obstruction [25]. Collagen provides tensile strength, but overaccumulation can inhibit contractility and the conduction of electrical impulses through the urinary organ walls [26]. A previous study [9] reported the histological analysis of the developing bladder and revealed that the smooth muscle, collagen and connective tissue are similar between genders during the second gestational trimester in human fetuses.

In an elegant paper Babu [27] compared interstitial cells of Cajal and collagen-to-muscle ratio between ureterovesical obstruction; ureteropelvic junction obstruction and human fetal ureters showed severe smooth muscle disarray with excess collagen. The fetal ureter segment had significantly less Interstitial Cells of Cajal (ICC) and significantly more collagen [27]. In this study, the authors found that the pathological changes at ureteropelvic junction and ureterovesical segments resemble fetal ureter morphology. The maturational process of human fetal ureter involves differentiation of smooth muscles cells/interstitial Cells of Cajal to establish the peristaltic machinery required to functionally connect the ureter at both ends and the authors suggest

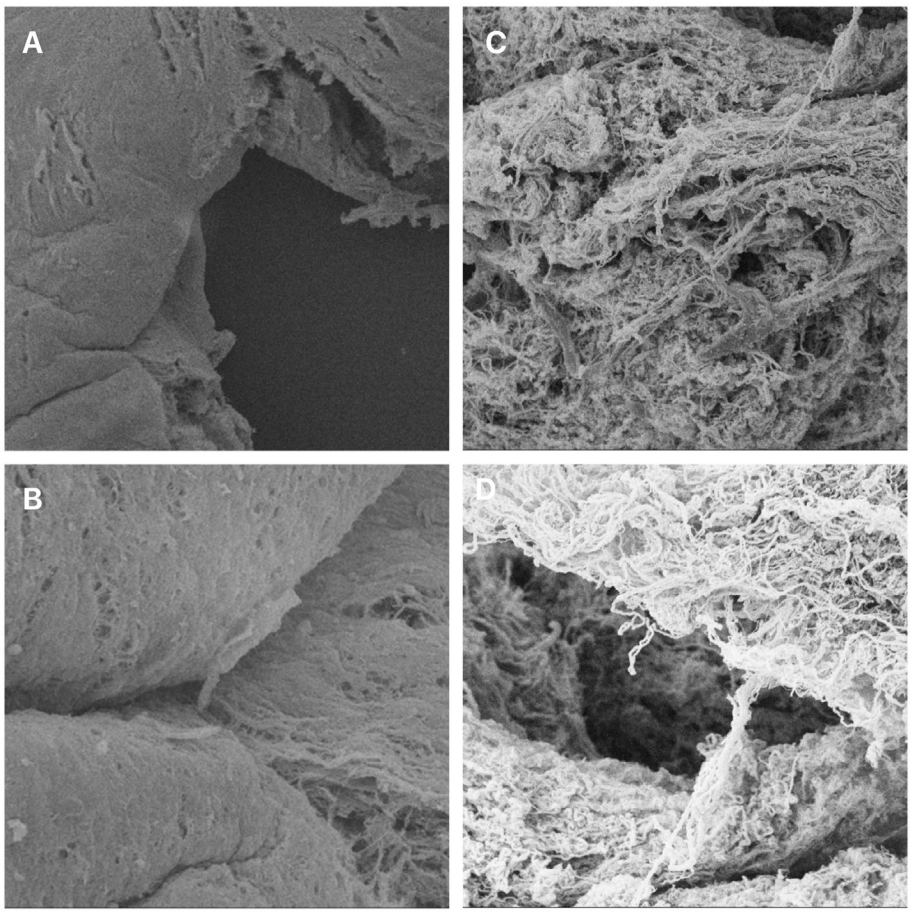

Fig. 4. Scanning electron microscopy (SEM) of the collagen system the fetal renal pelvis. (A) Renal pelvis sample of a male fetus 21 weeks postconception (WPC) examined by SEM. Original ×1000. (B) Renal pelvis sample of the same male fetus with 21 WPC. (C) Renal pelvis sample of a male fetus 17WPC examined by SEM. Original ×10,000. (D) Renal pelvis sample of a female fetus with 17WPC. Original ×10,000. In this preparation, the collagen fibers are visible. They are densely packed in parallel undulating arrays.

that the failure of this process, results in ureteral obstruction [27]. In our study, the qualitative analysis by SEM did not reveal differences in the distribution of collagen in the fetal renal pelvis between the sexes.

Our study included a significant number of male and female fetuses from the second gestational trimester. Besides the musculature, we analyzed the connective tissue, collagen and elastic fibers of the fetal renal pelvis. We did not observe the presence of elastic fibers. Some articles have shown that the ureter does not have elastic fibers during the human fetal period [3,28]. This may indicate that this extracellular matrix component appears only in the third gestational trimester in the fetal renal pelvis. We observed a positive correlation between the connective tissue and age in male fetuses. In older fetus studied we had more connective tissue with statistical significance.

There are some reasons that could explain the differences between male and female fetuses. 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 sphincter in male fetuses [29]. Nevertheless, to date no study has demonstrated structural differences in the bladder wall between the sexes in the human fetal period [9,26], but Koerener [30], in an elegant study with 48 human fetuses with ages between 9 and 35 WPC, analyzed the bladder musculature by immunohistochemical techniques and observed significant differences in the distribution of this musculature between the sexes. In our opinion the SMC alterations in renal pelvis could be secondary to pressure alterations during the growth of the prostate during the fetal development, but more studies will be necessary to discover the reason of this difference, maybe an experimental study with artificial elevation of the urethral pressure will be the next step.

We did not observe significant differences in the distribution of collagen of the renal pelvis between sexes, but a significant decrease of SMC was observed in male fetuses and a significant decrease of connective tissue in female fetuses. When we compared the gestational age with SMC, we did not find any correlation in male or female fetuses, but when we studied the connective tissue compared to the gestational age, we observed a positive correlation in male fetuses. In an elegant paper, Dienlenc [31] showed that the increased wall tension in the obstructed ureter decreases perfusion and can result in histologic changes in SMC. Our findings show a significant decrease in SMC concentration in male renal pelvises. We can speculate that this structural difference could be a predisposing factor for the higher incidence of urinary pathologies in male fetuses. Obviously, new studies with larger samples would be necessary to confirm this hypothesis.

The present study is the first comparative analysis in the literature to show the structure of the renal pelvis in human fetuses in the second gestational trimester with scanning electron microscopy and stereological methods.

We should mention some limitations of this study: a) unequal WPC distribution — we did not have fetuses with more than 23 WPC, but the second trimester is the most important period for growth and development of the kidneys, renal pelvis and ureter; and b) we only performed qualitative analysis of collagen by SEM. The quantitative measurements of the elastic fibers and collagen of fetal renal pelvis tissue by SEM will be a next step in this investigation.

The renal pelvis presents significant structural differences between male and female fetuses. The renal pelvis in males had fewer smooth muscle cells and presented a positive correlation of connective tissue with age and the renal pelvis in female had less connective tissue without correlation with the age.

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