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Structural and Ontogenetic Study of the Urachus in Human Fetuses

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Key Words

Urachus · Bladder · Development · Urachal anomalies · Urachal patency · Human fetuses

Abstract

The objective of this work was to conduct an ontogenetic and structural study of the urachus. We studied 40 human fetuses (13–20 weeks post conception, WPC). The urachus was stained in Masson's trichrome, to quantify connective tissue and smooth muscle and to determine the urachal lumen area. Weigert's resorcin-fuchsin was used to observe elastic fibers, and picrosirius red and immunohistochemistry analysis were used to observe collagen. The images were captured with Olympus BX51 microscopy and an Olympus DP70 camera. The stereological analysis was done using the software Image Pro and Image J, to determine volumetric densities. For biochemical analysis, the collagen concentrations were expressed per milligram of dry tissue. Means were compared using the unpaired t test ($p < 0.05$). Quantitative analysis documented a statistically insignificant increase ($p = 0.1475$) in volumetric densities of smooth muscle in the urachus of males (23.02%), when compared with females (18.43%), and a statistically significant increase ($p = 0.0439$) in volumetric densities of connective tissue in the urachus of females, (67.64%) when compared with males (58.38%). Total collagen concentrations in the male (31,919–56.792 μg/mg, mean 45,656) and female fetuses (33,485–48,527 μg/mg, mean 42,308) did not differ significantly ($p = 0.5912$). At higher gestational ages, the urachal lumen area was smaller. In 13th WPC fetuses, the urachal lumen area was 16,301 μm^2 and in 17th WPC fetuses, the urachal lumen area was 1,676 μm^2. We determined that the urachal lumen was closed from the 17th WPC in all fetuses.

Introduction

The urachus is a tubular structure between the bladder dome and umbilicus derived from obliteration of the allantoides [Stephens et al., 2002]. After birth, the urachus varies from 3 to 10 cm in length and from 8 to 10 mm in diameter [Sulak et al., 2008]. It is a 3-layered tubular structure, the innermost layer being lined with transitional epithelium, the middle layer composed of connective tissue, and the outermost muscular layer is in continuity with the detrusor muscle [Choi et al., 2006].

Abbreviations used in this paper	
95% confidence interval	95% CI
WPC	weeks post conception

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Between the 4th and the 5th month of fetal development, the bladder, which is located next to the umbilical region, migrates to the pelvis and is positioned next to the pubis [Stephens et al., 2002]. During bladder migration, the urachal lumen narrows and progressively closes, becoming a fibrous cord that connects the bladder dome to the umbilicus [Stephens et al., 2002]. After birth, this vestigial structure receives the name of median umbilical ligament [Ashley et al., 2006]. The time of urachal lumen obliteration and its dimensions are unknown.

Urachal anomalies are rare, with an incidence of 1:5,000 births, being more common in males, and usually detected at birth [McCrystal et al., 2001; Okegawa et al., 2006]. Urachal anomalies can be associated with other genitourinary anomalies including vesicoureteral reflux, crossed renal ectopia and hypospadias. The most common urachal anomalies are total patency of the urachus with umbilical fistula, partial patency of the urachus, cystic dilatation and urachal diverticula [McCrystal et al., 2001; Stephens et al., 2002].

In the adult population, malignant urachal tumors are rare and emerge predominantly from the epithelium, the most frequent being adenocarcinoma [Nascimento et al., 2004]. Men are affected by urachal cancer twice as frequently as women, and about 33% of the cases occur in patients <55 years of age [Ashley et al., 2006].

Knowledge of the structure and development of the urachus is important for the understanding of embryonic urinary tract drainage. Before the cloacal membrane ruptures, the mesonephric duct opens into the cloaca and probably transports urine produced by the mesonephros from the beginning of week 5 of gestation [Gobet et al., 1998]. During this period, the patent urachus may serve as a temporary urinary outlet. Specific studies on urachus development in human fetuses are scarce [Begg, 1930].

The objective of this work is to present an ontogenetic and structural study of the urachus in normal human fetuses, evaluating the difference between males and females during the human fetal period.

Material and Methods

The present work received institutional review committee and parent approval. This work was carried out in accordance with the ethical standards of the responsible institutional committee on human experimentation.

We studied 40 urachi obtained from 40 human fetuses (20 male and 20 female) that died of causes not related to the genitourinary tract. The fetuses were macroscopically well preserved and there was no evidence of congenital malformation. The gestational age of the fetuses was determined in weeks post conception (WPC), according to the foot length criterion. Presently, the foot length criterion is considered the most acceptable parameter used to calculate the gestational age [Hern, 1984; Mercer et al., 1987; Plat et al., 1988; Favorito et al., 2004]. The fetuses were also evaluated regarding crown-rump length and body weight immediately before dissection, and all measurements were taken by the same observer.

After the measurements, the fetuses were carefully dissected with the aid of a stereoscopic lens with 16/25× magnification. The fetal bladder was carefully removed with kidneys and ureters. The bladder dome, with the urachus and umbilical arteries, was fixed in 10% buffered formalin. The bladder dome was routinely processed for paraffin embedding, and 5-μm thick sections were obtained at 200-μm intervals. Urachal structural components, smooth muscle, connective tissue, elastic system fibers and collagen were studied by histochemical, immunohistochemical and biochemical methods.

Sections were stained with hematoxylin-eosin to assess the integrity of the tissue. We performed the following staining: Masson's trichrome to quantify connective tissue and smooth muscle and to determine the urachal lumen area, Weigert's resorcin-fuchsin with previous oxidation to observe elastic system fibers, and picrosirius red with polarization to observe different collagen types.

Connective tissue, smooth muscle and elastic system fibers were quantified by stereological method. Five sections were stained, and 5 fields of each section were selected. All selected fields were photographed, and the images were captured with Olympus BX51 microscopy and an Olympus DP70 camera. Images were transferred to the software Image Pro. The fibers were quantified using the software Image J to determine the volumetric density of each component (fig. 1a). The urachal lumen area was determined by the contour of the epithelium (fig. 1b).

The immunohistochemistry analysis of the collagen type III [mouse monoclonal collagen III (FH-7A) ABCAM] fibers used the avidin-biotin method with positive and negative controls. The slides were previously treated with poly-L-lysine for better adherence of the sections.

For the biochemical analysis of the collagen, tissue samples were fixed in acetone. The concentration of total collagen in the urachal tissue was determined by a colorimetric hydroxyproline assay. Thus, 5–14 mg of dry, defatted urachal tissue was hydrolyzed in 6 N HCl for 18 h at 118°C, as previously described [Cabral et al., 2003]. The assay was then carried out in the neutralized hydrolysates using a chloramine-T method [Bergman and Loxley, 1963]. Results were expressed as micrograms of hydroxyproline per milligram of dry, defatted tissue. The biochemical analysis was done with 11 urachus (5 female and 6 male).

Means were statistically compared using the unpaired t test ($p < 0.05$) with Graph Pad Prism software.

Results

The fetuses studied ranged in age between 13 and 20 WPC, weighed between 60 and 455 g, and had a crown-rump length between 7.3 and 19.3 cm (table 1).

Table 1. Age and fetal parameters of our sample

Fetus	Age, WPC	Weight, g	CRL, cm
1 M	17	300	17.3
2 F	20.4	455	19.3
3 M	16.4	245	16.5
4 M	15.3	125	13.3
5 F	13.7	120	12.2
6 F	13	60	9.5
7 M	20	400	18.5
8 F	19.5	285	18.5
9 M	18	365	18.5
10 F	17.4	280	16
11 M	18	280	16
12 M	17.3	280	17
13 F	16.4	155	14
14 M	16.2	230	15.5
15 F	16.5	220	16
16 M	18.2	300	15
17 F	17	295	16.4
18 F	16.2	215	16.1
19 M	17.8	350	17.7
20 F	19.3	300	18.9
21 F	18	300	16.5
22 F	16.1	200	16
23 F	16.6	225	16
24 F	14.5	105	12.5
25 F	18.6	335	16.5
26 F	17.8	280	15.5
27 M	14.7	165	13
28 M	15.5	190	13
29 M	17.5	245	15
30 M	16.6	150	14.5
31 F	17.4	290	16
32 F	18.4	350	17
33 M	16.6	185	15
34 M	15.9	185	14.5
35 F	18.2	405	18
36 M	18.5	145	15.5
37 M	17.6	190	16
38 M	14.5	90	12
39 F	19.4	400	18
40 M	16.4	220	15

The fetuses studied ranged in age between 13 and 20 WPC, weighed between 60 and 455 g, and had a crown-rump length between 7.3 and 19.3 cm.
M = Male; F = female; CRL = crown-rump length.

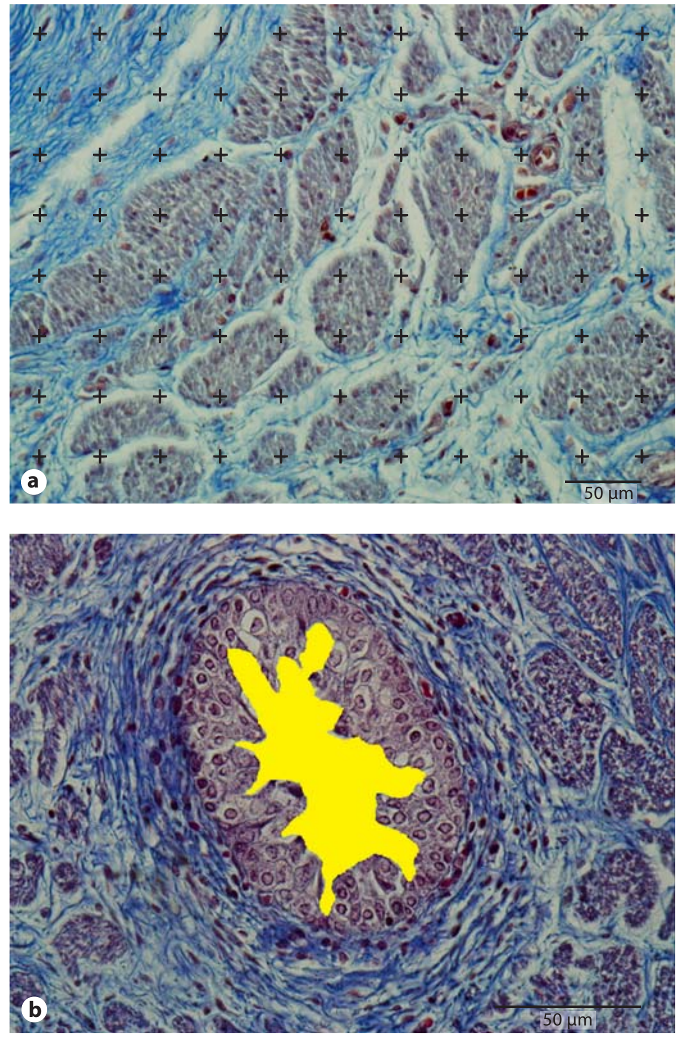


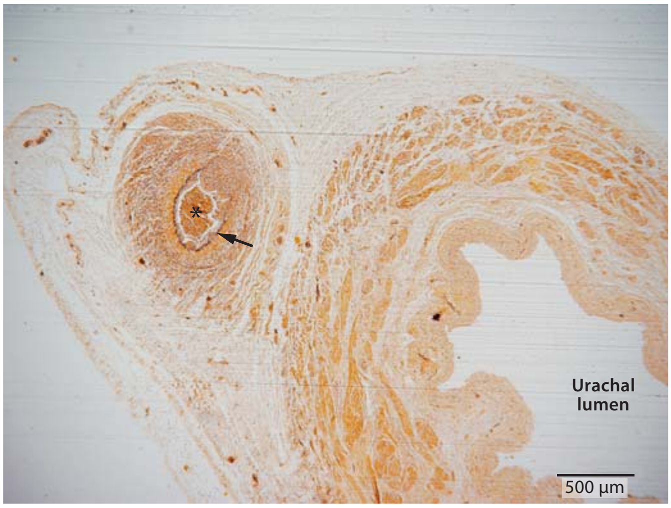
Fig. 1. Photomicrographies of a fetal urachus showing the morphometric analysis. **a** Quantification of smooth muscular cells of the urachus in a fetus at 15 WPC using the software Image J Test grid. Masson's trichrome. **b** Urachal lumen area measurement (yellow) in a fetus at 14 WPC using the software Image J. Masson's trichrome.

Elastic System Fibers

We did not observe elastic system fibers in any urachus analyzed. In figure 2, we can observe the urachus and umbilical arteries stained by Weigert's resorcin-fuchsin. Elastic system fibers were well visualized in the arterial wall, but were not observed in the urachus.

Connective Tissue

The urachus has a larger amount of connective tissue as compared with smooth muscle, both in males and females (table 2; fig. 3). Quantitative analysis documented a statistically significant increase ($p = 0.0439$) in volumetric density of connective tissue in the urachus of female fetuses [67.64%; 95% confidence interval (CI) 55.64–79.63] as compared with male fetuses (58.38%; 95% CI 34.97–61.81). When we compared the gestational age with connective tissue, we found a positive correlation only in females ($r = 0.9368$; 95% CI 0.8442–0.9751).



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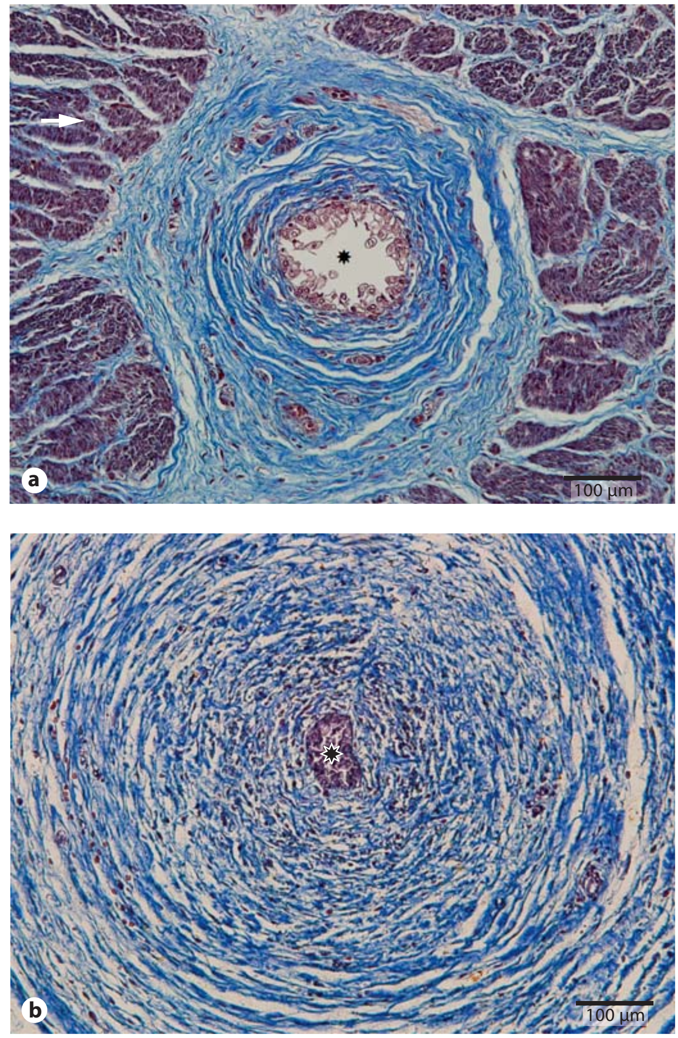

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Fig. 2. Photomicrography of a fetal urachus at 13 WPC in transversal section showing the elastic system fiber analysis. We can observe the urachus and its lumen, as well as the umbilical artery (*). There were no elastic system fibers in the urachal structure. These fibers were only observed in umbilical arteries (arrow). Weigert's resorcin-fuchsin with previous oxidation.

Fig. 3. Photomicrographies of a fetal urachus showing urachal smooth muscle in transversal section. **a** Great amount of smooth muscle (red) in a female fetal urachus at 15 WPC. Urachal lumen (*). Masson's trichrome. **b** Reduction in smooth muscle in a female fetal urachus at 20 WPC. Urachal lumen (*). Masson's trichrome.

In males, the correlation is very poor (r = 0.1846; 95% CI –0.2809 to 0.5799). At higher gestational ages, the amount of connective tissue was higher.

Smooth Muscle

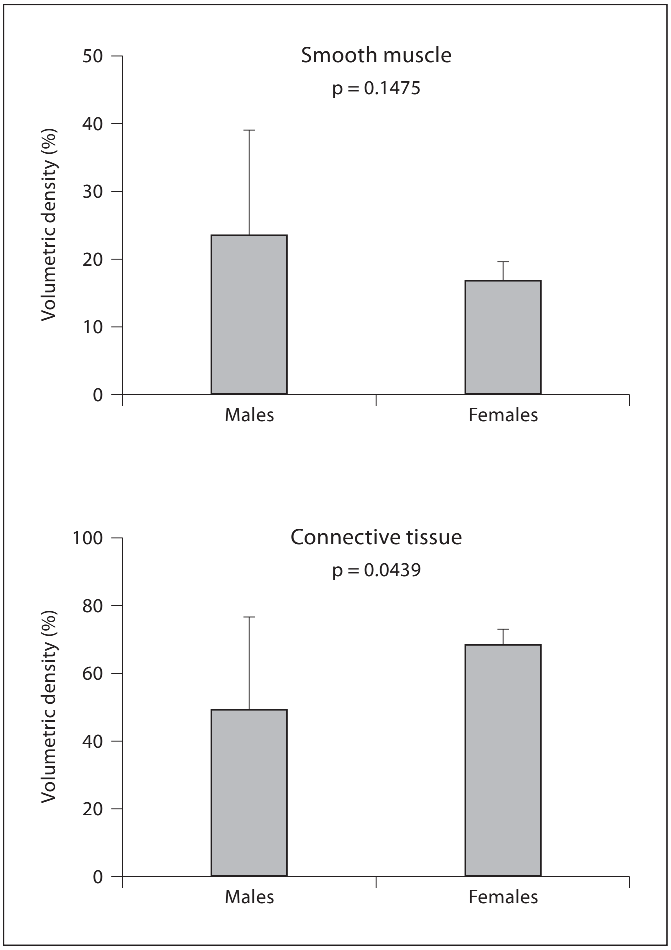
Quantitative analysis documented the volumetric density of smooth muscle cells in the urachus of males (23.02%; 95% CI 15.58–30.48) and females (18.43%; 95% CI 9.61–23.32), but there was no statistical significance (p = 0.1475; fig. 3). When we compared gestational age with smooth muscle, we observed that smooth muscle decreased in the older fetuses (female: r = –0.8280, 95% CI –0.9298 to –0.6083; male: r = –0.6324, 95% CI –0.8399 to –0.2635). However, we observed an inverse correlation only in females, indicating that the smooth muscle was smaller at greater gestational ages in female fetuses (fig. 4).

Table 2. Volumetric density (%) of connective tissue and smooth muscle in urachus in our sample

	Connective tissue	Smooth muscle
Males	58.38	23.02
Females	67.64	18.43
Total	63.01	20.72

Collagen

We observed a predominance of type III collagen (green in picrosirius red, and brown in immunohistochemistry) in younger fetuses, and in older fetuses, we observed a predominance of type I collagen (red in picro-



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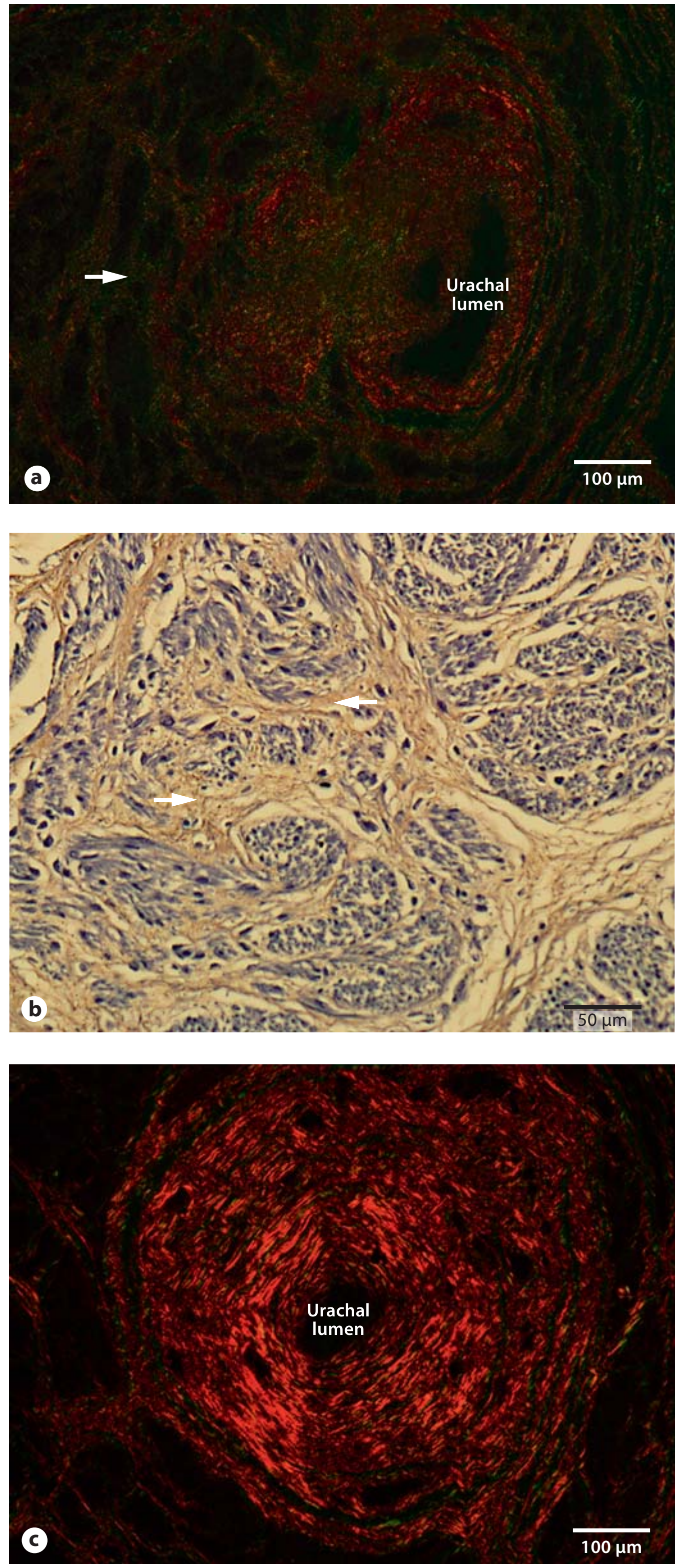

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Fig. 4. Comparative graphs between connective tissue and smooth muscle in male and female fetal urachus. There was more connective tissue in female than in male fetuses, and this difference was statistically significant ($p = 0.0439$). There was no difference in smooth muscle between male and female fetal urachus ($p = 0.1475$).

Fig. 5. Photomicrographies of a fetal urachus showing urachal collagen in transversal sections. **a** Predominance of green (white arrow), suggesting collagen type III presence in a fetus at 13 WPC. Picrosirius red with polarization. **b** Immunohistochemistry showing the type III collagen (brown, arrows) in a fetus at 15 WPC. Anti-collagen type III antibody. **c** Predominance of red, suggesting collagen type I presence in a fetus at 18 WPC. Picrosirius red with polarization.

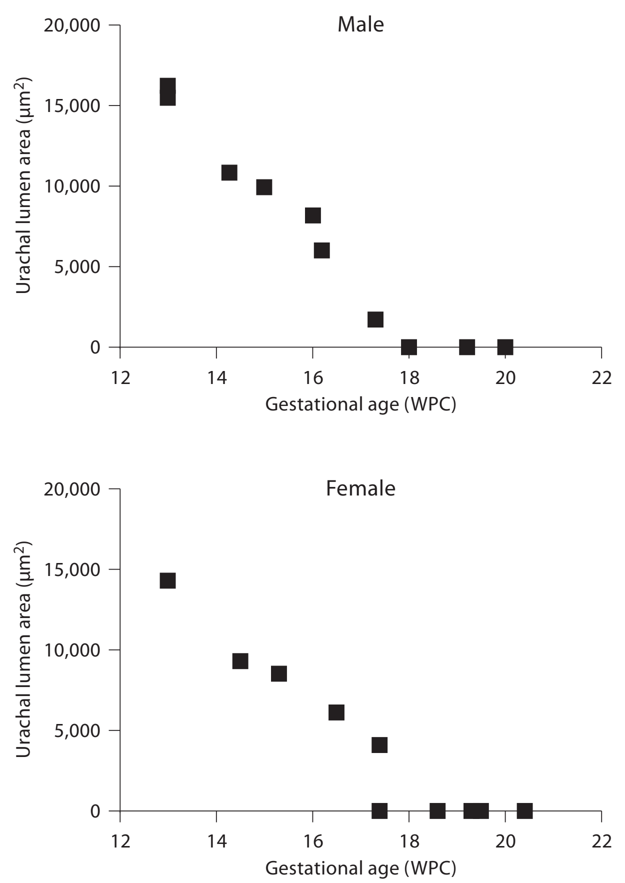

Fig. 6. Inverse correlation between gestational age and the urachal lumen area. The higher the gestational age, the smaller the urachal lumen area, which is obliterated as of the 17th WPC.

sirius red; fig. 5). Total collagen concentration in the urachus of males (45,656 μg/mg; 95% CI 35.46–55.86) and females (42,308 μg/mg; 95% CI 35.50–49.11) did not differ significantly ($p = 0.5912$). In female fetuses, total collagen concentration increases with age; however, in male fetuses, we did not observe an age correlation (female: $r = 0.9022$, 95% CI 0.009715–0.9936; male: $r = 0.1906$, 95% CI –0.7347 to 0.8680).

Urachal Lumen and Epithelium

When we compared the gestational age with the urachal lumen area, we found a negative correlation, both in males ($r = -0.9666$; 95% CI –0.9923 to –0.8611) and in females ($r = -0.9462$; 95% CI –0.9875 to –0.7832). At greater gestational ages, the urachal lumen area was smaller (figs. 6, 7a, b). The urachal lumen area varied from 1,676 (17th WPC) to 16,301 μm^2 (13th WPC). The urachal lumen was closed at the 18th WPC in both males and females.

The transitional epithelium in the urachus was clearly identified in fetuses until the 17th WPC, in which the urachal lumen was open (fig. 7c). In fetuses aged ≥18 WPC, the urachal epithelium was not visualized because the urachal lumen was closed (fig. 7d).

Discussion

The allantoides emerge on the 16th day of the embryonic period as a fine tubular structure derived from the yolk sac. The allantoides are continuous on one side with the ventral wall of the cloacae and on the other with the abdominal wall (umbilicus). The ventral portion of the cloacae develops into the bladder after its division by the genitourinary sinus, so initially, the bladder extends until the umbilicus [Stephens et al., 2002].

The exact moment of the urachal closing is controversial, supposedly occurring between the 10th and 20th WPC [Ashley et al., 2006; Okegawa et al., 2006; Yapok et al., 2008]. However, there are no references regarding at which gestational week the urachal lumen is obliterated. In our study, in fetuses <16 WPC, the urachal lumen was patent with an area >8,000 μm^2. All studied fetuses >17 WPC had the urachal lumen obliterated.

As of the 17th WPC, when the urachal lumen was closed, we observed a decrease in smooth muscle and an increase in type I collage in the urachus of both male and female fetuses. We observed another change in the transitional epithelium. No fetuses >17 WPC with an obliterated urachal lumen showed transitional epithelium. In younger fetuses, the transitional epithelium was clearly shown throughout the urachal extension. These structural alterations suggest a tissue alteration which leads to a fibrotic tissue.

Collagen and elastic fibers are the fibrotic components of the extracellular matrix and are related to pathological alterations in different tissues. The greenish fibers that appear under the picrosirius stain characterize the prevalence of type III collagen, a newly formed collagen likely produced by muscular retraction, which suggests an intense tissue turnover [Cavalcanti et al., 2007].

In our sample, we observed a predominance of type III collagen in younger fetuses and a predominance of type I collagen in fetuses with obliterated lumen. This result

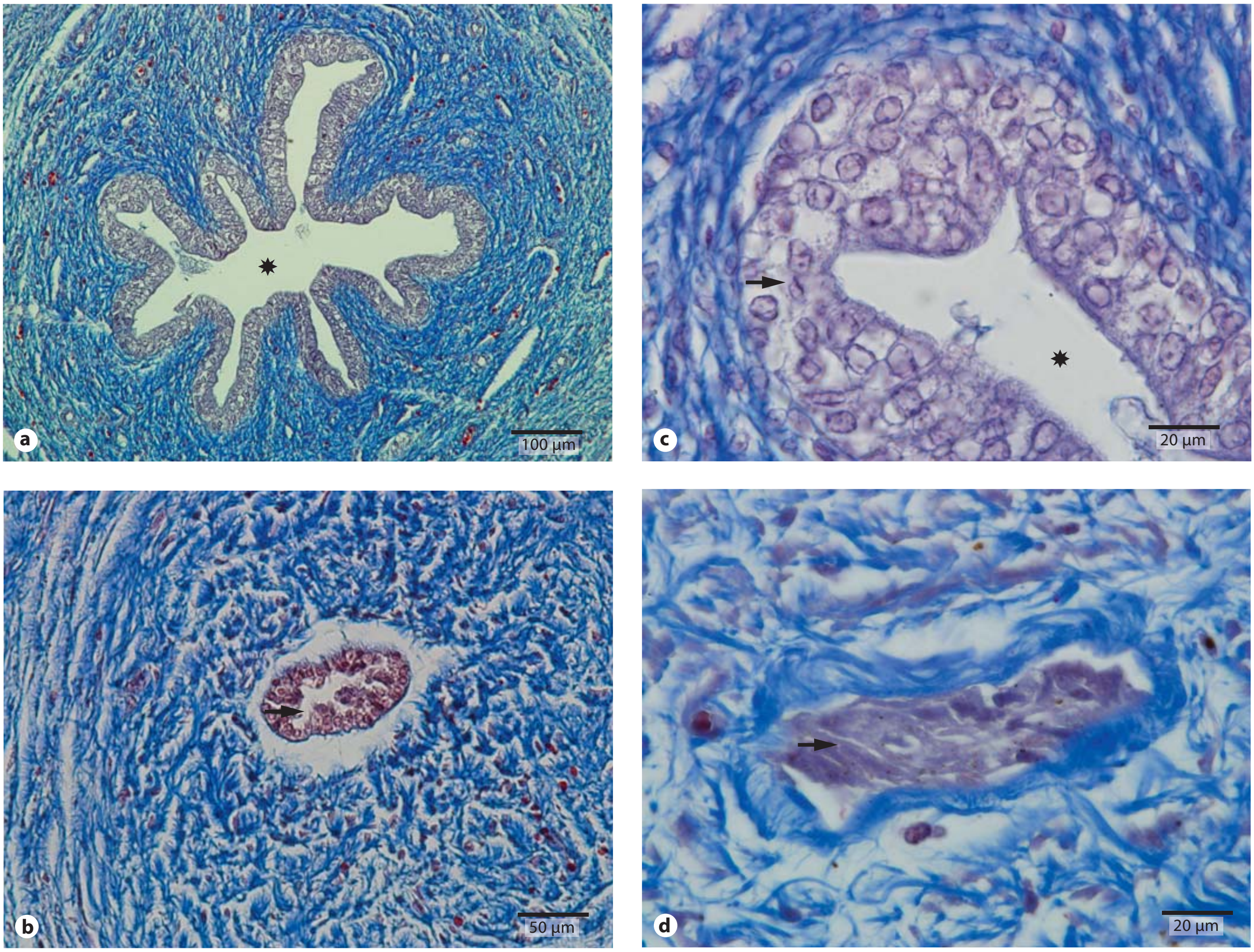

Fig. 7. Photomicrographies of the fetal urachal lumen and epithelium in transversal sections. **a** Urachal patent lumen (*) in a fetus at 14 WPC. Masson's trichrome. **b** Urachal lumen (arrow) is closing in a fetus at 17 WPC. Masson's trichrome. **c** Transitional epithelium (arrow) of the urachus in a fetus at 14 WPC. Masson's trichrome. **d** Urachal lumen completely obliterated (arrow) and without transitional epithelium in a fetus at 20 WPC. Masson's trichrome.

confirms that a great tissue alteration occurred in the fetal bladder before urachal lumen closing. Through biochemical quantification, we observed an increase in total collagen concentrations in older female fetuses. We did not observe a positive correlation between total collagen concentration and gestational age in male fetuses.

Elastic system fiber alterations are involved in fibrotic tissue formation; however, we did not observe in our samples the presence of elastic fibers in the urachus. This may indicate that this extracellular matrix component appears only in the third gestational trimester in the fetal bladder. Previous studies showed the elastic system fibers in other human fetal genitourinary organs [Bastos et al., 2004].

Bastos et al. [2004] observed scarce and fine elastic system fibers in the homogeneous and intense cellular tissue of the corpus spongiosum in a fetus at 15 weeks of gestation; in a fetus at 36 weeks of gestation, the trabeculae of the corpus spongiosum delimitating large vascular spaces was noticed. The elastic system fibers are plentiful and organized in older fetuses [Bastos et al., 2004]. This study indicates that the elastic system fibers in the genitourinary fetal system are more evident and developed in the third gestational trimester. Our sample was composed of fetuses in the second gestational trimester, probably the period where elastic system fibers are still forming in the fetal bladder.

Although rare, urachal anomalies are more prevalent in males than in females [Cilento et al., 1998; Nascimento et al., 2004; Choi et al., 2006; McCrystal et al., 2007]. In this study, we observed some structural differences in the fetal urachus between sexes at the same gestational age. The most relevant structural difference is in the connective tissue. The amount of connective tissue in female fetuses is significantly higher than in male fetuses. Furthermore, a positive correlation between connective tissue and gestational age was observed only in females. This suggests that at greater gestational ages, the amount of connective tissue in female fetuses is higher. This was not observed in male fetuses.

It is difficult to speculate if the smaller amount of connective tissue in male fetuses could explain the higher incidence of urachal anomalies in male patients. Structural studies about the amount of connective tissue in male and female patients with urachal pathologies would be necessary to confirm this hypothesis.

Male urethral development is complete by weeks 12–13 of gestation, at which time fetal urine has been produced for approximately 6 weeks. This overlap seems to be a critical point in urinary tract function and drainage since the other possible urinary outlet of the bladder during this time is the urachus, which connects the bladder with the allantois. If the urachus is already closed, this increased bladder outlet resistance may be the beginning of fetal hydronephrosis. In this paper, we confirmed that all fetuses with more than 17 WPC and no evidence of congenital malformation had the urachal lumen obliterated. Interestingly, the prune belly syndrome is associated with a patent urachus at birth in 50% of cases [Lattimer, 1958] in contrast to bladder outlet obstruction due to posterior urethral valves, in which urachal patency is uncommon [Kaefer et al., 1995].

Conclusion

Urachal lumen closing occurs in the 17th WPC. After the lumen closing, we notice an absence of the transitional epithelium in its interior, a decrease in the amount of smooth muscle and an increase in type I collagen, which indicates a characteristic remodeling in fibrous tissue formation. We do not observe elastic fibers in the urachus during the human fetal period analyzed. The urachal structure is similar in both males and females with more connective tissue in female fetuses. The data obtained in the present study can be used as basic knowledge related to the development of the urachus and embryonic urinary tract drainage.

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