Journal of Pediatric Surgery 55 (2020) 2221–2225

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Study of prostate growth in prune belly syndrome and anencephalic fetuses

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ARTICLE INFO

Article history:
Received 15 August 2019
Received in revised form 10 October 2019
Accepted 23 October 2019

Key words:
Prostate
Prune belly syndrome
Anencephaly
Human fetuses

ABSTRACT

Background: To compare the growth of the prostate in anencephalic, prune belly syndrome (PBS) and control fetuses.
Methods: We studied 35 prostates from normal human fetuses aged 11–22 weeks postconception (WPC); 15 from anencephalic fetuses aged 13–19 WPC; and 6 from PBS fetuses aged 13–31WPC. After prostate dissection, we evaluated the prostate length, width and thickness with the aid of a computer program (Image Pro and Image J). The fetal prostate volume (PV) was calculated using the ellipsoid formula: PV = [length × thickness × width] × 0.523. The prostates were dissected and the PV was measured with the aid of the same computer program. Means were statistically compared using the unpaired t-test and linear regression was performed.
Results: In 2 PBS fetuses we observed prostatic atresia. We did not observe significant differences in PV when comparing the control group (PV: 6.1 to 313.81 mm, mean = 70.85 mm: SD = 71.43 mm) with anencephalic fetuses: $p = 0.3575$ (PV: 5.1 to 159.11 mm, mean = 42.94 mm; SD = 40.11 mm) and PBS fetuses: $p > 0.999$ (PV: 10.89 to 148.71 mm, mean = 55.4 mm; SD = 63.64 mm). The linear regression analysis indicated that the PV in the control group ($r^2 = 0.3096$; $p = 0.0004$), anencephalic group ($r^2 = 0.3778$; $p = 0.0148$) and PBS group ($r^2 = 0.9821$; $p < 0.009$) increased significantly and positively with fetal age ($p < 0.0001$).
Conclusions: We did not observe significant differences in development of the prostate in fetuses with anencephaly and in 2/3 of fetuses with PBS during the fetal period studied. In 1/3 of the PBS fetuses, the prostate had important atresia.
Level of evidence: Level III.

The prostate develops from the urogenital sinus in response to testosterone stimulation by the fetal testis production, which starts at 8 weeks of gestation [1,2]. The growth of the prostate begins in the 10th gestational week and accelerates starting in the second trimester, associated with the fetal testosterone production during this period [2,3].

Anencephaly and prune belly syndrome (PBS) are rare and severe anomalies. Anencephaly is the most severe fetal NTD, resulting from failure of the neural tube to close at the base of the skull in the third or fourth week (day 26 to 28) after conception, leaving the skull bones that usually surround the head unformed [4]. Anencephaly is observed in 0.03% of all births. It occurs at a rate three to four times higher in female fetuses compared to males [5]. PBS is a disorder characterized by deficiency or hypoplasia of the abdominal muscles and malformations of the urinary tract, such as large and hypotonic bladders, dilated and tortuous ureters and bilateral cryptorchidism [6,7].

Urethral obstruction occurs in one-third of patients with PBS and can be the primary cause of the bladder alterations in this syndrome [8]. Studies of the development of the prostate during the human fetal period in PBS and anencephalic fetuses are rare. Previous studies have shown the growth of the prostate during the human fetal period [9]; the ontogeny of the extracellular matrix of fetal prostate [10]; the prostatic ductal budding [11]; and the arrangement of the muscle fibers of the striated urethral sphincter and its relationship with the prostate during the fetal period in humans [12]. But to our knowledge, there are no reports comparing the prostate volume development among normal, PBS and anencephalic fetuses during the human fetal period.

Our hypothesis was that the PBS and anencephaly alter the volume of the human fetal prostate. The aim of this paper is to compare the growth of the prostate from anencephalic and PBS fetuses and compare it to the parameters of controls during the second gestational trimester.

1. Methods

1.1. Study population

This study was carried out in accordance with the ethical standards of the hospital's institutional committee on human experimentation. (IRB: 2.475.334, CAAE: 78881317.4.0000.5259).

We studied 35 normal human fetuses aged 11–22 weeks postconception (WPC); 15 anencephalic fetuses aged 13–19 WPC; and 6 PBS

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https://doi.org/10.1016/j.jpedsurg.2019.10.054
0022-3468/

fetuses aged 13–31 WPC during the period from July 2016 to December 2018. The fetuses came to our laboratory as a donation from the obstetric section of our hospital. The fetuses of control group were macroscopically well preserved, showed no signs of malformations and the demise was 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 [13–15]. The fetuses were also evaluated regarding crown-rump length (CRL) and body weight immediately before dissection. The same observer made all the measurements.

After the measurements, the fetuses were carefully dissected with the aid of a stereoscopic lens with 16/25× magnification. The prostate was removed together with the ureters, bladder and genital organs. After dissection, we evaluated the following measurements: prostate length, prostate width and prostate thickness, with the aid of a computer program (Image Pro and Image J) (Fig. 1). The prostate volume (PV) was calculated using the ellipsoid formula: PV = [length × thickness × width] × 0.523 [9]. In two cases of PBS we observed severe obstruction in prostatic urethra. We also observed prostatic atresia in two fetuses with PBS. These two cases of PBS were excluded because of the impossibility of performing prostatic measurements.

We analyzed the histology of the PBS with prostatic atresia. The prostate was separated from the other structures and was fixed in 10% buffered formalin, and routinely processed for paraffin embedding, after which 5-μm thick sections were obtained at 200-μm-intervals. The sections were stained with hematoxylin–eosin to assess the integrity of the tissue. We also performed staining with picrosirius red and Masson's trichrome.

1.2. Statistical analysis

All parameters were statistically processed and graphically described. The Shapiro–Wilk test was employed to ascertain the normality of the data and to compare quantitative data between normal vs. anencephalic fetuses, while the Kruskal–Wallis test was used to assess gender differences. Simple linear correlations (LC) were calculated for prostate volume according to fetal age, weight and crown-rump length.

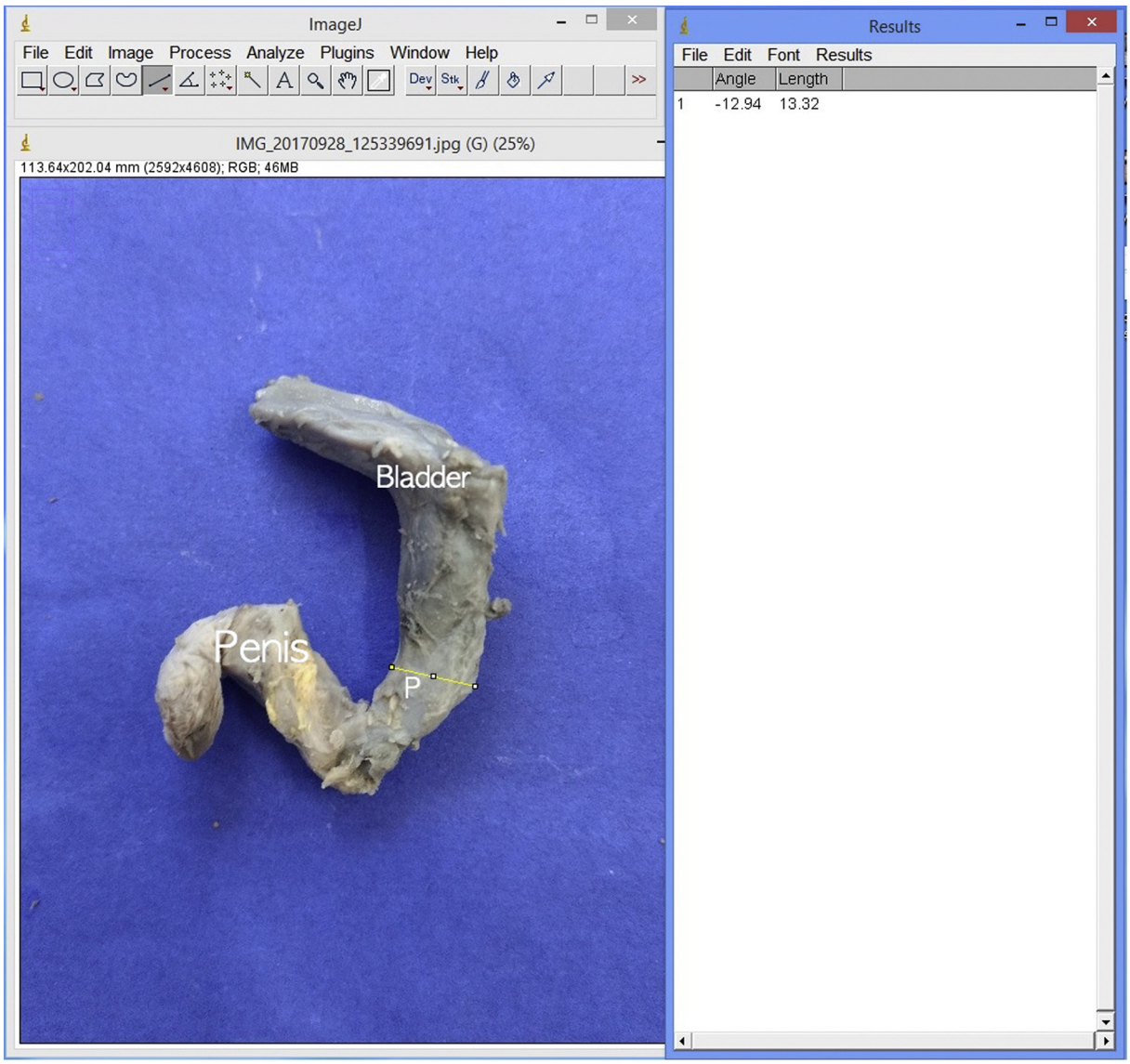

Fig. 1. The figure shows the prostate measurements with the aid of the computer program Image Pro and Image J. We can observe the genital organs of a fetus of the control group with 17 weeks postconception. The fetus was carefully dissected with the aid of a stereoscopic lens with 16/25× magnification and the genital organs were removed. The penis, prostate (P) and bladder can be observed in the figure. After the dissection, we evaluated the prostate length, width and thickness with the aid of a computer program (Image Pro and Image J). This figure shows the measurement of the prostate width with a yellow line.

The statistical analysis was performed with the Graphpad Prism (Version 6.01) software.

2. Results

The fetuses presented weights between 16 and 525 g and had crown-rump lengths between 6.5 and 20.5 cm. The summary of the findings regarding the fetal age, weight, crown-rump length and prostatic measurements is shown in Table 1. In the two PBS fetuses with prostatic atresia, we observed that the prostatic urethra had a significantly smaller lumen (Fig. 2).

Table 1
The table shows the fetal parameters analyzed in the 54 fetuses studied.

Fetus	Age (WPC)	Anomaly	Weight (g)	CRL (cm)	PL	PW	PT	PV
1	10.8	none	16	6.5	1.59	2.91	2.59	6.27
2	11	none	28	8	3.79	1.98	3.31	13.01
3	11.1	none	20	7	3.91	2.71	3.12	17.31
4	11.7	none	30	9.5	2.51	2.47	1.88	6.1
5	12	none	36	9	4.46	2.23	3.85	20.05
6	12.8	none	76	10.5	3.89	2.97	2.38	14.4
7	12.9	none	94	13.5	3.43	3.21	3.23	18.62
8	13.3	none	68	13	3.28	2.4	2.97	12.24
9	13.9	none	104	12	2.39	2.36	2.46	7.27
10	14.5	none	134	14	7.38	5.04	5.28	102.83
11	15	none	196	15	4.18	3.91	3.71	31.75
12	15.1	none	188	16.5	5.03	4.48	5.48	64.66
13	15.2	none	168	16.5	5.63	2.88	3.72	31.58
14	15.3	none	206	15	5.41	3.39	3.43	32.94
15	15.4	none	124	13.5	3.85	2.83	3.54	20.2
16	15.5	none	116	14	6.94	5.65	5.18	106.35
17	15.7	none	232	15.5	6.17	4.65	5.19	77.97
18	16	none	188	15	4.39	3.87	3.68	32.74
19	16.3	none	145	16	6.36	4.48	3.66	54.6
20	16.3	none	198	17	5.71	5.2	4.01	62.34
21	16.3	none	272	17	5.84	4.35	4.27	56.8
22	16.4	none	238	16	11.08	9.18	5.58	297.18
23	16.7	none	162	17	6.32	4.41	3.76	54.5
24	17	none	202	15.5	5.13	5.22	5.26	73.75
25	17	none	242	18	6.36	4.92	5.09	83.39
26	17.6	none	265	17	4.94	4.67	4.74	57.26
27	17.6	none	312	18.5	7.52	5.82	4.09	93.73
28	17.6	none	330	17	4.35	4.73	4.56	49.13
29	17.6	none	370	19	7.17	3.87	4.46	64.8
30	18.5	none	365	19	10.26	8.54	6.84	313.8
31	20	none	390	17	6.82	6.15	3.26	71.59
32	20	none	525	20.5	6.96	6.47	6.77	159.63
33	21	none	350	17	6.35	6.12	6.29	127.99
34	21	none	450	20	6.92	5.12	6.20	115.02
35	22	none	436	19	6.85	5.31	5.86	111.6
36	13.3	Anenc	32	10	3.07	1.19	3.15	6.03
37	13.5	Anenc	68	10.5	6.25	4.09	3.96	53.0
38	13.8	Anenc	52	7.5	5.24	1.92	2.27	11.96
39	14	Anenc	54	8.5	2.87	3.12	3.36	15.75
40	14.5	Anenc	72	10	2.49	1.77	2.21	5.1
41	14.8	Anenc	94	12	3.86	3.03	2.94	18.0
42	14.9	Anenc	82	12	5.82	2.44	3.3	24.54
43	14.9	Anenc	96	11	3.6	3.44	2.94	19.06
44	15.3	Anenc	96	10	5.32	3.83	3.3	35.21
45	16	Anenc	110	11	5.47	5.67	4.32	70.15
46	16	Anenc	132	10	5.41	4.9	4.55	63.15
47	16.3	Anenc	206	14	6.73	8.44	5.35	159.11
48	16.5	Anenc	138	10.5	4.97	4.74	4.42	54.52
49	16.7	Anenc	142	12	5.75	2.87	3.32	28.69
50	18.8	Anenc	248	14	5.9	4.82	5.36	79.81
51	12.9	PBS	86	10.5	3.82	2.58	2.11	10.89
52	13.1	PBS	65	10	6.1	2.68	2.26	19.35
53	13.2	PBS	65	11	5.17	2.87	5.49	42.65
54	15.7	PBS	210	14	8.31	4.65	7.35	148.71

In 2 cases of Prune Belly Syndrome we observed prostatic atresia and the fetuses were excluded from the measurements. Fetal age in weeks postconception (WPC); weight in grams (g); crown-rump length (CRL) and total length in centimeters (cm). We can also observe the prostatic measurements. PL = Prostate length; PW = Prostate width; PT = Prostate thickness and PV = prostatic volume.

We did not observe significant differences between PV when compared the control group (PV: 6.1 to 313.81 mm, mean = 70.85 mm: SD = 71.43 mm) with anencephalic fetuses: $p = 0.3575$ (PV: 5.1 to 159.11 mm, mean = 42.94 mm; SD = 40.11 mm) and PBS fetuses: $p > 0.999$ (PV: 10.89 to 148.71 mm, mean = 55.4 mm; SD = 63.64 mm).

The linear correlation comparing PV data and fetal anthropometry was assessed (Fig. 3). Although all the correlations were positive, it must be said that r^2 values less than 0.4 reflect very weak correlation, while r^2 between 0.4 and 0.7 reflects moderate correlation and r^2 greater than 0.7 indicates strong correlation. The linear regression analysis indicated that the PV in the control group ($r^2 = 0.3096$; $p = 0.0004$), anencephalic fetuses ($r^2 = 0.3778$; $p = 0.0148$) and PBS group ($r^2 = 0.9821$; $p < 0.009$) increased significantly and positively with fetal age ($p < 0.0001$).

The linear regression analysis indicated that the PV in the control group ($r^2 = 0.3626$; $p < 0.0004$) and anencephalic group ($r^2 = 0.5872$; $p < 0.0009$) increased significantly and positively with fetal weight. The linear regression analysis indicated the PV in the PBS group did not increase significantly with fetal weight ($r^2 = 0.8957$; $p = 0.0536$).

The linear regression analysis also indicated that the PV in the control group ($r^2 = 0.3055$; $p = 0.0009$), anencephalic group ($r^2 = 0.4021$; $p = 0.0111$) and PBS group ($r^2 = 0.9720$; $p = 0.0141$) increased significantly and positively with crown rump length ($p < 0.0001$).

Finally, the linear regression analysis indicated that the PV in the control group ($r^2 = 0.3307$; $p = 0.0005$), anencephalic group ($r^2 = 0.6269$; $p = 0.0004$) and PBS group ($r^2 = 0.4809$; $p = 0.0176$) increased significantly and positively with total length ($p < 0.0001$).

3. Discussion

The development of the prostate during the second gestational trimester is strongly associated with the production of testosterone and dihidrotestosterone (DHT) [1–3]. The prostate ductal budding starts at 10 weeks of gestation and continues at least through 14 weeks, when multiple epithelial outgrowths invade the surrounding mesenchyme [11,16]. The growth of the prostate during the second gestational trimester leading to the occurrence of a transient obstruction below the bladder was noted in previous studies [17,18]. The prostate is enlarging between 10 and 20 weeks, which is exactly coincidental with the onset of urinary production from the kidneys that starts about 10 weeks. Therefore, in fetuses with PBS and an enlarged bladder this will lead to potential compression of the prostate, which would normally be responding to androgen stimulation to grow.

The etiology of PBS is controversial. Some authors suggest that prune belly syndrome may arise from either anatomic obstruction of various types or functional obstruction from megacystis [8]. Volmar [19] analyzed 11 cases of PBS and observed mechanical obstruction in 8 cases. Stephens [8] described a significant sample of 21 babies with PBS and observed important alterations in the prostate and posterior urethra. In that sample, the authors observed that the prostate gland was indistinguishable macroscopically from the surrounding urethral wall in 19 of the 21 fetuses, while in our sample, in 4 of the 6 fetuses with PBS (66.6%) it was possible to clearly identify the urethral wall. Stephens [8] reported that the prostate was mainly fibrous in 8 of the 21 fetuses studied.

Previous studies about the bladder and ureter [20,21] in anencephalic fetuses have demonstrated significant alterations in the structure of these two organs. Lesions in the nervous system with consequent alteration in nerve regulation could be a plausible hypothesis to explain structural changes in the bladder and ureter in fetuses with neural tube defects. The prostate stroma has several components like blood vessels, lymphatic and nerves [3]. The growth of the prostatic parenchyma during human fetal development is induced by neuroendocrine cells, neural components and prostate fibroblasts [3].

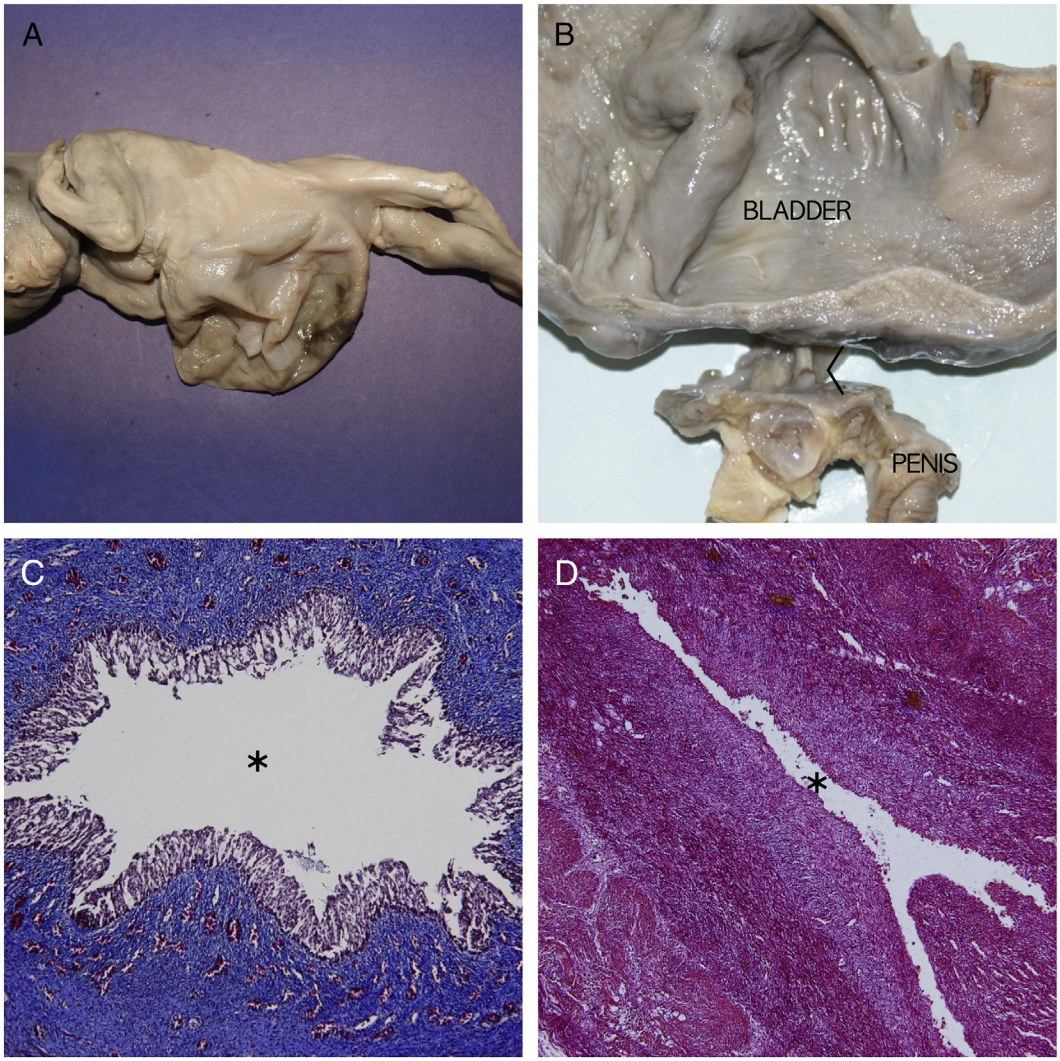

Fig. 2. Prostatic atresia in Prune Belly Syndrome (PBS). (A) Fetus with 17 weeks postconception with PBS, showing the characteristic aspect of the abdominal wall. (B) The urinary and genital organs were dissected and the enlarged bladder was opened. The prostatic atresia in this fetus can be observed (arrowhead - <). (C) Photomicrograph showing the urethral lumen (*) of a fetus of the control group with 17 WPC. Masson's trichrome ×100. (D) Photomicrograph showing the urethral lumen (*) of the PBS fetus with 17 WPC. The evident diminution of the urethral lumen can be seen in this case. Picrosirius red ×100.

Prostate nerves in anencephalic fetuses might be modified due to cerebral lesions, with consequent brain control damage in pelvic nerves. However, in the present study, macroscopically we did not observe significant differences in the prostate of the anencephalic fetuses compared with the control group. Further research with investigation of the distribution of prostatic nerves in anencephalic fetuses will be necessary to confirm or refute the existence of prostatic structural alteration in these severe anomalies.

We did not observe significant alterations in prostate growth in the anencephalic and PBS groups when compared to the control group. The prostate volume had significant correlations with fetal age, total length, crown-rump length and weight in anencephalic fetuses. In PBS fetuses, the prostate volume had a significant correlation with all fetal measurements except weight. In the PBS group, we did not observe significant alterations in prostate growth, but in two fetuses we observed prostatic atresia. According to Stephens [8], about 1/3 of PBS fetuses had urethral or prostatic atresia. In our small sample, we observed the same ratio of this obstructive factor. PBS fetuses can have several profiles of urethral and prostatic anatomy [22]. Our observations confirm this finding: fetuses with prostatic urethral atresia have a bad prognosis that precludes development. In cases where the prostate does not have significant changes, the chances of reaching term are probably greater.

This paper is the first in the literature to report the PV correlations with fetal parameters in anencephalic and PBS fetuses. We should mention some limitations of this study: a) Small sample size. PBS and anencephalic fetuses are rare, so observations on a small sample may be important, although the small number is a weakness. b) Unequal WPC distribution between PBS fetuses and the two other groups. This is a weakness but the two PBS fetuses with more than 17 WPC had prostatic atresia and were removed from the morphometric analysis of the prostate. c) The measurements of the prostate were only performed on fetuses during the second gestational trimester, because of the great difficulty to obtain fetuses with more than 500 g in our country. This is a limitation, but this period is the most important for prostatic development. d) The histologic analysis of all the prostates studied for technical reasons was not done. This is also a weakness, but will be addressed in the next step of this research program.

We did not observe significant differences in development of the prostate in fetuses with anencephaly and in 2/3 of fetuses with prune belly syndrome during the human fetal period studied. In 1/3 of PBS

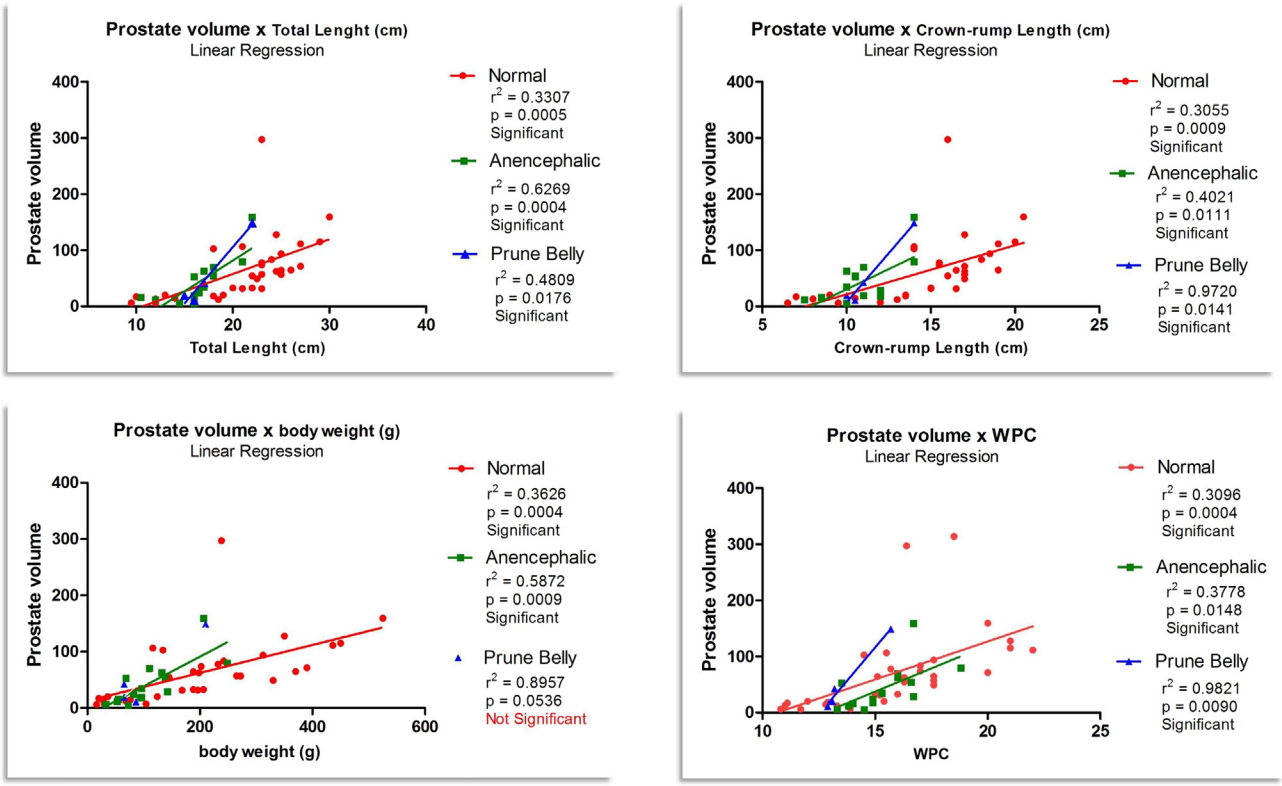

Fig. 3. Correlation of the prostate volume of normal, PBS and anencephalic fetuses with fetal age, fetal weight; crown-rump length (CRL) and total length during the fetal period studied. The points plotted represent the mean values obtained for each week studied. (A) Total length (cm). The linear regression analysis indicated that the prostate volume in the control group ($r^2 = 0.3307$; $p = 0.0005$), anencephalic group ($r^2 = 0.6269$; $p = 0.0004$) and PBS group ($r^2 = 0.4809$; $p = 0.0176$) increased significantly and positively with total length ($p < 0.0001$); (B) Crown rump length – CRL (cm). The linear regression analysis indicated that the prostate volume in the control group ($r^2 = 0.3055$; $p = 0.0009$), anencephalic group ($r^2 = 0.4021$; $p = 0.0111$) and PBS group ($r^2 = 0.9720$; $p = 0.0141$) increased significantly and positively with crown rump length ($p < 0.0001$); (C) AGE (WPC). The linear regression analysis indicated that the prostate volume in the control group ($r^2 = 0.3096$; $p = 0.0004$), anencephalic group ($r^2 = 0.3778$; $p = 0.0148$) and PBS group ($r^2 = 0.9821$; $p < 0.009$) increased significantly and positively with fetal age ($p < 0.0001$) and (D) Weight (g). The linear regression analysis also indicated that the prostate volume in the control group ($r^2 = 0.3626$; $p < 0.0004$) and anencephalic group ($r^2 = 0.5872$; $p < 0.0009$) increased significantly and positively with fetal weight. Finally, the linear regression analysis indicated prostate volume in the PBS group did not increase significantly with fetal weight ($r^2 = 0.8957$; $p = 0.0536$).

fetuses, the prostate had important atresia. Further studies with larger samples should be performed to confirm our findings.

Funding

This work was supported by the National Council for Scientific and Technological Development (CNPq Brazil) (Grant number: 301522/2017-0) and the Rio de Janeiro State Research Foundation (FAPERJ) (Grant number: E-26/202.873/2017).

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