ORIGINAL

Ultrasonographic assessment of testicular volume in Japanese newborn infants

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Abstract : Purpose : It is essential to determine normative data of testicular size and penile length in newborn infants in order to assess the development during the fetal period. In this study, we evaluated testicular volumes using ultrasonography and also measured penile lengths in Japanese newborn infants. We also examined the associations of the development of these organs with factors including placental weight and anthropometric data. Methods : One hundred and fifteen full-term male newborn infants were recruited for this study. Testicular size was measured by ultrasonography. Results : Mean testicular volumes were 187.4 mm^3 in the right testis and 185.4 mm^3 in the left testis, and there was no significant difference between the right and left testicular volumes. Right and left testicular volumes showed positive and significant correlations with body lengths and weights. Testicular volumes in the right and left sides were not significantly associated with penile lengths. In 4 groups according to gestational weeks, testicular volume increased significantly with advance of gestational weeks in the left testis. Conclusion : We determined testicular volumes using ultrasonography in Japanese newborn infants. We believe that the results for newborn infants provide important information for assessment of the development of fetal testicular volume. J. Med. Invest. 69 : 256-260, August, 2022

Keywords **:** *testicular volume, penile length, newborn infants, placenta, ultrasonography*

INTRODUCTION

Increases in the prevalences of undescended testis and hypospadias have been reported (1, 2). It has been suggested that testicular size and circulating inhibin B level are involved in impaired perinatal testicular development (3). Thus, studies on the development of male genital organs such as testicular size and penile length and on factors related to development from the fetal period to neonatal period are important.

Assessment of testicular volume has been conducted by using an orchidometer and by ultrasonography. For newborn infants whose testicular sizes were small, it was shown that ultrasonography provides more accurate volumes than those obtained by an orchidometer (4-6). There have been several studies in which ultrasonography was used to assess the development of testicular size. It has been reported that mean testicular volumes in newborn infants ranged from 1.55 cm^3 to 2.44 cm^3 (7, 8). Testicular volume has been reported to steadily increase until 5-6 months of age (8) and then decrease up to 9 months of age (9). In addition, a positive correlation between testicular volume at 3 months of age and serum inhibin B concentration, which is a marker of Sertoli cell function, has been reported (3). On the other hand, there have been some reports on the association between testicular volume and penile length (10, 11). Since the occurrence of undescended testis and the occurrence of hypospadias might be related to endocrinological circumstances during the fetal period, measurements of penile length as well as testicular volume are needed. However, there have been few studies in which measurement of testicular size by ultrasonography and measurement of penile length were performed simultaneously.

It has been reported that descent of the testes into the scrotum through the inguinal canals begins at around 26 weeks of gestation and that both testes are present in the scrotum by 32 weeks of gestation in most cases (12). It has also been reported that testicular diameter measured by ultrasonography in fetuses between 20 and 40 weeks of gestation showed a significant and positive correlation with gestational age (13). Recently, moderate testicular growth in fetuses aged 10-22 weeks post-conception was demonstrated (14). It is necessary to determine normative data of testicular size in newborn infants in order to clarify the changes during the fetal period as a longitudinal study. In Japan, there are few data for testicular sizes in newborn infants. In addition, factors that are involved in testicular and penile development have not been clarified. Studies on these factors as well as evaluations of testicular size and penile length are important since testicular development during the perinatal period may influence reproductive health in adulthood. In this study, testicular volumes were measured using ultrasonography and penile lengths were also measured in Japanese newborn infants. In addition, the associations of the development of these organs with factors including placental weight and anthropometric data were examined.

MATERIALS AND METHODS

One hundred and fifteen full-term male newborn infants who were born by a normal delivery process and had a normal process after birth in our hospital were recruited for this study. Newborn infants born in multiple births, newborn infants with a fetal growth restriction, newborn infants with asphyxia or an anomaly,

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and newborn infants who did not have a normal process after birth were excluded. Also, newborn infants who had maternal complications including severe hypertensive disorders of pregnancy and severe threatened premature delivery that required admission to the hospital were excluded.

Testicular sizes were measured by ultrasonography (ARIETTA 65LE linear probe manufactured by HITACHI, frequency of 5-18 MHz) as shown in Fig 1. Sagittally, we measured length (mm) and width (mm) and transversally we measured height (mm). The epididymis is not included in the volume measurement. Testicular volume was calculated using the following formula : length/2 x width/2 x height/2 x $4\pi/3$. The stretched penile length was measured from the tip of the glans penis to the pubic ramus in a flaccid state by using a transparent plastic ruler when maintaining normal temperature in a quiet state and in the supine position. All measurements were performed by the same experienced investigator. The ethics committee of our hospital reviewed and approved the study (approval number 3433).

Statistical analyses

Differences in sizes of the right testis and left testis were analyzed by the Mann-Whitney U test. Correlations of testicular volume with body length, body weight, placental weight and penile length were analyzed by using Spearman's rank correlation test. Differences in testicular volume, body length, body weight, placental weight and penile length in each week during the 10-month period of gestation were analyzed by the Kruskal-Wallis test and post-hoc analysis was performed by using the Bonferroni test.

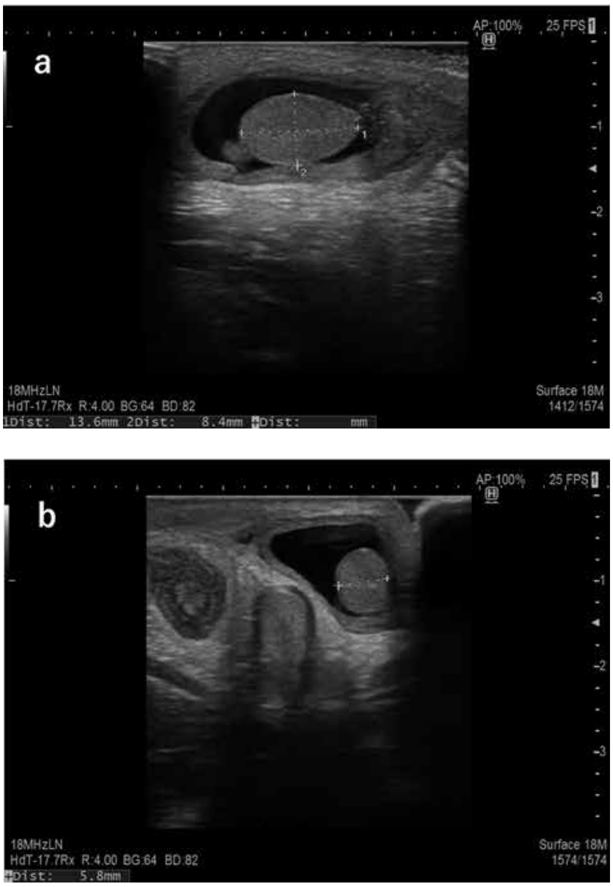

Fig 1. Ultrasonographic measurements of testicular size.
a. Sagittal plane : Arrows indicate length (1) and width (2) of the testis.
b. Transverse plane : Arrow indicates height of the testis.

RESULTS

To assess the reliability of measurements of testicular sizes by ultrasonography, measurements in 8 randomly selected newborn infants not included in this study were performed prior to initiation of the study. Intra-class correlation coefficients of length in the right testis and left testis were 0.927 and 0.908, respectively. Intra-class correlation coefficients of width in the right testis and left testis were 0.759 and 0.758, respectively. Intra-class correlation coefficients in height in the right testis and left testis were 0.862 and 0.768, respectively.

The background characteristics of newborn infants and their mothers are shown in Table 1. The mean (± standard deviation : SD) gestational period was 38.7 (± 1.1) weeks and the median gestational period was 39 weeks. Mean (± SD) body length was 49.7 (± 1.6) cm, mean (± SD) body weight was 3045.1 (± 337.4) g and mean (± SD) placental weight was 570.1 (± 91.5) g.

Axes (length, width and height), cross-sectional areas and volumes in the right testis and left testis are shown in Table 2. Mean (± SD) testicular volumes were 187.4 (± 60.2) mm^3 in the right testis and 185.4 (± 56.5) mm^3 in the left testis. Median testicular volumes were 176.8 mm^3 in the right testis and 178.6 mm^3 in the left testis. There were no significant differences in the three-way axes, cross-sectional areas and volumes between the right testis and left testis. Mean (± SD) penile length was 25.2 (± 3.5) mm and median penile length was 25.0 mm.

Correlations of testicular volumes with body length, body weight, placental volume and penile length are shown in Table 3. Testicular volumes in the right testis and left testis showed positive and significant correlations with body lengths ($r=0.230$, $p=0.014$ and $r=0.279$, $p=0.003$, respectively) and body weights ($r=0.281$, $p=0.002$ and $r=0.311$, $p<0.001$, respectively). Testicular volumes in the right testis and left testis were not significantly associated with placental weights and penile lengths.

We divided the newborn infants into four groups according to gestational weeks (37w0d-37w6d, 38w0d-38w6d, 39w0d-39w6d

Table 1. Background characteristics of newborn infants and their mothers

Body length (cm)		49.7 ± 1.6
Body weight (g)		3045.1 ± 337.4
Placental weight (g)		570.1 ± 91.5
Penile length (mm)		25.1 ± 3.6
Apgar score		8.1 ± 0.9 (1 min)
		9.3 ± 0.5 (5 mins)
Gestational week (week)		38.8 ± 1.1
Method of delivery	Vaginal delivery	79 (68.7%)
	Cesarean section	36 (31.3%)
Maternal information		
Age (years)		34.5 ± 5.3
Parity	0	63 (54.8%)
	1	37 (32.2%)
	≥2	15 (13.0%)
Height (cm)		158.3 ± 5.8
Weight (kg)		56.4 ± 11.6
Body mass index (kg/m^2)		22.5 ± 4.3
Number of smokers	Before pregnancy	4
	Current	0

mean ± standard deviation

and 40w0d-41w6d) and analyzed significant differences (Table 4). Body lengths and body weights increased significantly with advance of gestational weeks during the 10-month period. Testicular volumes increased significantly with advance of gestational weeks in the left testis (p=0.039) but not in the right testis (p=0.101). There was no significant difference in testicular volumes between the right testis and the left testis in any of the four groups. Placental weight and penile length did not change significantly with advance of gestational weeks during the 10-month period.

DISCUSSION

In the present study, we determined testicular volumes in Japanese healthy newborn infants using ultrasonography. We clarified that intra-class correlation coefficients in testicular sizes were more than 0.7, suggesting that measurement of testicular size by ultrasonography has high reliability. Nguyen *et al*. reported that intra-class correlation coefficients were greater than 0.80 with minima of 0.57 (8). Main *et al*. reported that intra-observer variation was 16-25% and interobserver variation was 24-27% (3). Ultrasonography is thus a useful tool for measurement of testicular volume in newborn infants.

An orchidometer is widely used in clinical practice. It has been reported that mean testicular volumes measured using an orchidometer varied from 1.1 to 2.5 cm^3 in newborn infants (11, 15). Goede *et al*. reported that testicular volumes measured by the Prader orchidometer showed an accurate goodness of fit with ultrasonography measurement (16). Sakamoto *et al*. reported that the correlation between testicular volume calculated by the three ultrasound formulas and actual testicular volume determined by water displacement was stronger than the correlation between testicular volume measured by an orchidometer and actual volume in patients scheduled for bilateral orchiectomy (17). The use of an orchidometer has been shown to overestimate testicular volume because the epididymis is included in the volume (5). This overestimation is especially pronounced in newborn infants in whom the epididymis is large relative to the size of the testis than in older boys (18). It has been suggested that ultrasonography provides more accurate volumes than those obtained by an orchidometer, especially in small testes (4-6). Studies using

Table 2. Testicular sizes in the right testis and left testis

		Right testis	Left testis	P value
Length (mm)	Mean (± SD)	9.54 (± 1.15)	9.38 (± 1.09)	0.439
	Median (10-90 percentile)	9.50 (8.3-10.9)	9.40 (8.04-10.8)	
Width (mm)	Mean (± SD)	6.20 (± 0.84)	6.23 (± 0.83)	0.624
	Median (10-90 percentile)	6.10 (5.10-7.36)	6.20 (5.14-7.20)	
Height (mm)	Mean (± SD)	5.92 (± 0.94)	5.94 (± 0.77)	0.848
	Median (10-90 percentile)	5.90 (4.78-7.16)	5.90 (5.10-7.0)	
Cross-sectional area (mm^2)	Mean (± SD)	46.7 (± 9.4)	46.1 (± 9.6)	0.702
	Median (10-90 percentile)	46.5 (35.1-59.5)	46.0 (35.3-57.8)	
Volume (mm^3)	Mean (± SD)	187.4 (± 60.2)	185.4 (± 56.5)	0.929
	Median (10-90 percentile)	176.8 (117.7-275.3)	178.6 (123.8-256.3)	

SD : standard deviation

Table 3. Correlations of testicular volumes with body height, body weight, placental weight and penile length

			Body length	Body weight	Placental weight	Penile length
Testicular volume	Right	r	0.230	0.281	0.150	-0.088
		p	0.014	0.002	0.109	0.348
	Left	r	0.279	0.311	0.150	-0.038
		p	0.003	< 0.001	0.108	0.684

Table 4. Differences in testicular volume, body length, body weight, placental weight and penile length in each week during the 10-month period of gestation

	37w0d-6d	38w0d-6d	39w0d-6d	40w0d-41w6d	P value
Number	15	39	29	32	
Body length (cm)	48.2 (± 1.2)	49.5 (± 1.1)	49.7 (± 1.5)	50.6 (± 1.6)	< 0.001
Body weight (g)	2714.7 (± 228.6)	3001.0 (± 259.2)	3069.4 (± 316.4)	3231.6 (± 361.2)	< 0.001
Placental weight (g)	569.9 (± 61.6)	560.1 (± 85.6)	561.5 (± 85.1)	590.3 (± 113.8)	0.569
Penile length (mm)	24.2 (± 3.8)	24.4 (± 3.6)	25.9 (± 3.0)	25.8 (± 3.8)	0.209
Testicular volume (right)	163.0 (± 59.4)	199.9 (± 58.6)	179.8 (± 62.5)	190.3 (± 58.6)	0.101
Testicular volume (left)	154.2 (± 52.3)	191.8 (± 45.5)	183.5 (± 58.3)	193.8 (± 65.6)	0.039

ultrasonography have shown that mean testicular volumes in newborn infants were 170 mm^3 in the USA (8), 98 (66-288) mm^3 in Finland, 95 (46-207) mm^3 in Denmark (3), 300 ± 100 mm^3 in the USA (7) and 270 ± 20 mm^3 in the Netherlands (9). In the present study conducted in Japan, mean testicular volumes were 187.4 mm^3 in the right testis and 185.4 mm^3 in the left testis. Thus, testicular volumes differ slightly among studies. Testicular volume may be different depending on the ethnicity, although no racial difference in testicular sizes in African-American and European-American neonates has been reported (7).

It has been reported that no differences were found between the left testicular volumes and right testicular volumes in healthy boys aged from 1 to 69 months (9) and in children and adolescents aged from 0 to 17 years (15). In fetuses aged 10-22 weeks post-conception, there was no significant difference in mean testicular volumes between the right testis and left testis (14). In the present study, there was no significant difference in testicular volume between the right testis and left testis. On the other hand, Kaplan *et al*. reported that mean volume in the right testis was significantly larger than that in the left testis in healthy full-term neonates aged 0-3 days, but they suggested that the difference was unlikely to be clinically relevant since testes are bilaterally symmetric in their anatomy with the exception of venous drainage (7). The relationship between testicular volume and function in the left testis and right testis may be different in newborn infants and adults. In men who requested fertility testing, it has been reported that right testicular volume in the control group was significantly larger than that in men with oligoasthenozoospermia, although left testicular volume was not different between the control group and men with oligoasthenozoospermia (19). Anatomically, the right testicular vein directly inserts into the inferior vena cava and provides less flow turbulence and back pressure, but the left testicular vein inserts into the left renal vein at a right angle. Thus, it has been suggested that the right testis may have a natural advantage and that its function is more powerful than that of the left testis (19).

In the present study, testicular volumes were significantly associated with body weights and body lengths in newborn infants. Main *et al*. reported that body length and body weight were correlated to testicular volumes at 3 and 18 months of age but not at birth (3). By using an orchidometer, Ting *et al*. found a significant association between testicular volume and body weight in Malay and Chinese infants but not in Indian infants (11). Mondal *et al*. reported that there was a moderate positive correlation between testicular volume measured by an orchidometer and body weight or body length (10). Newborn infants with a large body length and large body weight may have large testicular volumes.

We showed that both right and left testicular volumes were not significantly correlated with placental volume. Placental volume has been reported to be approximately one sixth of birth weight (20). A balanced growth between the fetus and placenta is important for fetal development. It has been suggested that the ratio of placental weight to birth weight can be used as a predictor for placental functional efficiency (21). Eskild *et al*. reported that placental weight and the ratio of placental weight to birth weight were higher in pregnancies with infant Apgar scores $\leqq 7$ than in pregnancies with Apgar scores >7 (22). The placenta includes endocrine function as well as transport capacity and metabolic function. Since information on the relationship between intrauterine placental volume and birth weight is limited, the relationship between testicular volume and endocrine function in the placenta should be examined.

It has been reported that mean penile length in newborn infants varied from 23 mm to 36 mm in many races (10, 11, 15, 23, 24). In our study, mean penile length was 25.1 mm. Penile length may be different according to ethnicity. We did not find a significant correlation between testicular volume and penile length. Ting *et al*. also reported that there was no significant association between penile length and testicular volume measured by an orchidometer (11). However, Mondal *et al*. reported that there was a positive moderate correlation between penile length and testicular volume measured by an orchidometer and that there was a positive moderate correlation between penile length and body weight or body length (10). Further studies on the associations of endocrinological factors with testicular volume and penile length may be important.

We divided the 10-month gestation period into four duration groups in order to determine whether testicular volume increases or not during the 10-month period of gestation. We showed that there is a significant increase in left testicular volume but not in right testicular volume with advance of gestational weeks, although body weights and body lengths significantly increased. The reason for the difference in increases in testicular volumes in the left and right testes is not clear, but that inconsistency may be attributable to the small number of infants in each gestational group, especially those with 37w0d-6d of gestation. Since both testes are present in the scrotum in most cases by 32 weeks, the difference in changes in the right and left testicular volumes for not only full-term newborn infants but also fetuses after 32 weeks of gestation might be important.

To our knowledge, there have been few studies on changes in testicular volumes in fetuses. In a morphometric study, it was shown that testicular volumes in fetuses with anencephaly aged 13-19 weeks post-conception did not increase with fetal age (25). Recently, a significant correlation of testicular growth with fetal parameters in fetuses aged 10-22 weeks post-conception has been reported (14). It has been hypothesized that some of problems in adulthood may be attributable to testicular dysgenesis in fetal life (26, 27). It has been reported that anogenital distance, which is hormonally determined during fetal life, is associated with testicular volume, penile length and seminal parameters in men aged 18-19 years (28). Follow-up studies are needed to determine whether differences in early postnatal testicular volume are predictive of adult reproductive health in humans.

This study was a cross-sectional study. A longitudinal study on testicular development during gestation is needed. For measurement of testicular volume using ultrasonography, we used the formula length/2 x width/2 x height/2 x $4\pi/3$. There are several available equations including the Lambert equation, the Hansen formula and the prolate ellipsoid formula. However, there is no consensus on which is the most accurate in newborn infants. We excluded newborn infants whose mothers had complications such as severe hypertensive disorders of pregnancy and severe threatened premature delivery. Main *et al*. reported that the presence of maternal gestational diabetes showed a weak and positive association with testicular size at 3 months of age (3). Evaluation of the relationships between degrees of maternal complication such as gestational diabetes and testicular volume may be needed. As well as studies on the interaction between fetal testicular development and intrauterine body growth, further studies on circulating endocrinological factors in the cord, maternal blood and amniotic fluid that are involved in testicular development are needed.

CONCLUSION

In conclusion, we determined testicular volumes using ultrasonography in Japanese newborn infants. We believe that the results for newborn infants provide important information for assessment of the development of fetal testicular volume measured by ultrasonography.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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ETHICAL STATEMENTS

This study was approved by the Institutional Review Board of Tokushima University and was conducted according to the principles of the Declaration of Helsinki.

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