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Ultrasonographic Study of Testicular Development in Beetal Bucks

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ABSTRACT

Two dimensional (2D) ultrasound imaging of testis was conducted on six prepubertal bucks from 1 month to 9 months of age. Testicular scanning was done through direct contact in longitudinal and vertical positions by using 6.0 MHz frequency with a real time ultrasound scanner. The testicular parenchyma (TP) appeared homogenous with a coarse medium echo-pattern which appeared anechoic at first month of age and then moderately echogenic as development occurred with advancement of age. The mediastinum appeared as a longitudinal structure in the middle of testis having greater echogenicity than TP in longitudinal plane and as an echogenic spot in centre of TP in transverse plane. The scrotal septum was seen as a hyperechoic structure in lateral sonograms from initial scanning onwards. The tunics of the testes appeared as a bright echogenic line. Inter-testicular septal depression also appeared between testes as anechoic linear band on transverse scanning. The length, width and circumference of the testes showed significant increase ($P<0.05$) with the advancement of age. Pixel value of testes showed an incremental pattern with slight deviations at few points. All testicular parameters increased linearly as age advanced, with maximum increase from third to fourth month.

Keywords: Beetal, buck, scrotum, testes, ultrasound.

Ultrasound of the male reproductive system is the primary imaging method used to evaluate disorders of the testicles and surrounding tissues. Diagnostic ultrasonography is a valuable alternative imaging system that can provide more accurate information about reproductive disorders in comparison to traditional methods (Kahn, 1994). This technique presents an image of the internal architecture of soft tissues and could provide visualization of abnormal testicular and epididymal features, thus complementing the clinical examination.

The main functions of ultrasound are to evaluate anatomical structures and determine the echogenicity of testicular parenchyma (TP) and mediastinum (Chandolia *et al.*, 1997; Clark *et al.*, 2003). It can also be useful in monitoring progressive changes that occur in testis at different stages of maturation (Ahmad and Noakes, 1995). Increased echogenicity of TP in direct proportion with age has been reported (Brito *et al.*, 2004). According to Tapping and Cast (2008), testes of pre-pubertal animals had low to medium echogenicity, whereas testes of post-pubertal animals demonstrated medium homogeneous echogenicity.

Ultrasonographic imaging of testes has previously been done in bulls (Ahmed *et al.*, 2012), rams (Gouletsou and Fthenakis, 2010 and Andrade *et al.*, 2014), goats (Jeyakumar *et al.*, 2013) and other mammals (Pozor, 2005; Ball, 2008). However, despite the advantages of this diagnostic method, there is little information about testicular development using ultrasonography. Most of the work has been done in ram and other species, but limited information is available on ultrasonographic architecture of buck testis.

Keeping in view the importance of ultrasound in diagnostic and developmental studies in pre-pubertal bucks, the present study was planned to study the developmental changes of testes from birth to puberty (up to 9 months of age).

MATERIALS AND METHODS

Study area

The present study was conducted in the Department of Veterinary Gynaecology and Obstetrics with collaboration of Department of Animal Genetics and Breeding, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal sciences, Hisar (Haryana).

Animals

Ultrasound was done on six Beetal bucks starting from 1 month and carried up to 9 months of age. They were kept on grazing as well stall feeding.

Clinical examination

Initially, a general health examination was performed in the bucks. The scrotum was observed for the presence of lesions, testes were palpated, compressed and compared to each other for their size, shape and consistency. The testes were squeezed gently throughout to detect pain reaction. Free movement of the testes into the scrotum and lack of other tissue mass were confirmed.

Ultrasonographic imaging

Ultrasonographic imaging of the testes was carried out in bucks by laying them in lateral position on a table after clipping the hairs on scrotum. For ultrasonographic examination, advanced ultrasound machine (Toshiba Nemio XG) with 2D linear probe (3.5-8 MHz) transducer was used. After securing the animal, gel was applied directly on the scrotum and then imaging was done. Longitudinal and transverse planes were imaged for scanning of testes using linear probe. For imaging the longitudinal plane, mediastinum streak of each testis was taken as the landmark, while the dot mediastinum was taken as landmark for imaging the transverse planes. Length of testes was measured in longitudinal plane and width was measured perpendicular to the longitudinal plane (Jeyakumar *et al.*, 2013).

Assessment of Pixel values of the images

The setting of the machine was kept same for all the readings. The pixel values were calculated using Photoshop-7 (Adobe, USA) for each image of testis. The pixel values were average of three values taken from different areas of testis, keeping cursor size and area of observation same.

Table 1. Showing average (mean ± SE) of the length (L), circumference (C) and width (W) of left (LT) and right (RT) testis of Beetal bucks (n=6).

AGE (Months)	LLT(mm)	LRT(mm)	CLT(mm)	CRT(mm)	WLT(mm)	WRT(mm)
1	20.41 ±1.33^{a}	20.05 ±1.62^{a}	51.96 ±2.72^{a}	50.83 ±3.64^{a}	8.00 ±0.60^{a}	9.18 ±0.63^{a}
2	27.98 ±2.01^{ab}	27.41 ±1.23^{b}	67.25 ±4.25^{a}	68.15 ±3.49^{b}	11.26 ±0.95^{ab}	11.60 ±1.18^{ab}
3	33.88 ±2.58^{b}	33.36 ±1.86^{b}	85.15± 6.25^{b}	83.05 ±4.97^{b}	14.61 ±0.92^{b}	14.76 ±1.24^{b}
4	46.10 ±3.90^{c}	43.53 ±3.50^{c}	118.70 ±7.86^{c}	119.70 ±8.70^{c}	25.56 ±2.08^{c}	27.20 ±1.89^{c}
5					30.10 ±0.95^{d}	30.53 ±1.33^{cd}
6					32.38 ±1.39^{de}	31.58 ±1.33^{d}
7					33.55 ±1.34^{de}	32.66 ±1.18^{d}
8					34.61 ±1.17^{e}	33.83 ±1.16^{d}
9					35.51 ±1.10^{e}	34.43 ±1.17^{d}

Means with different superscript within the column are different significantly ($P<0.05$).

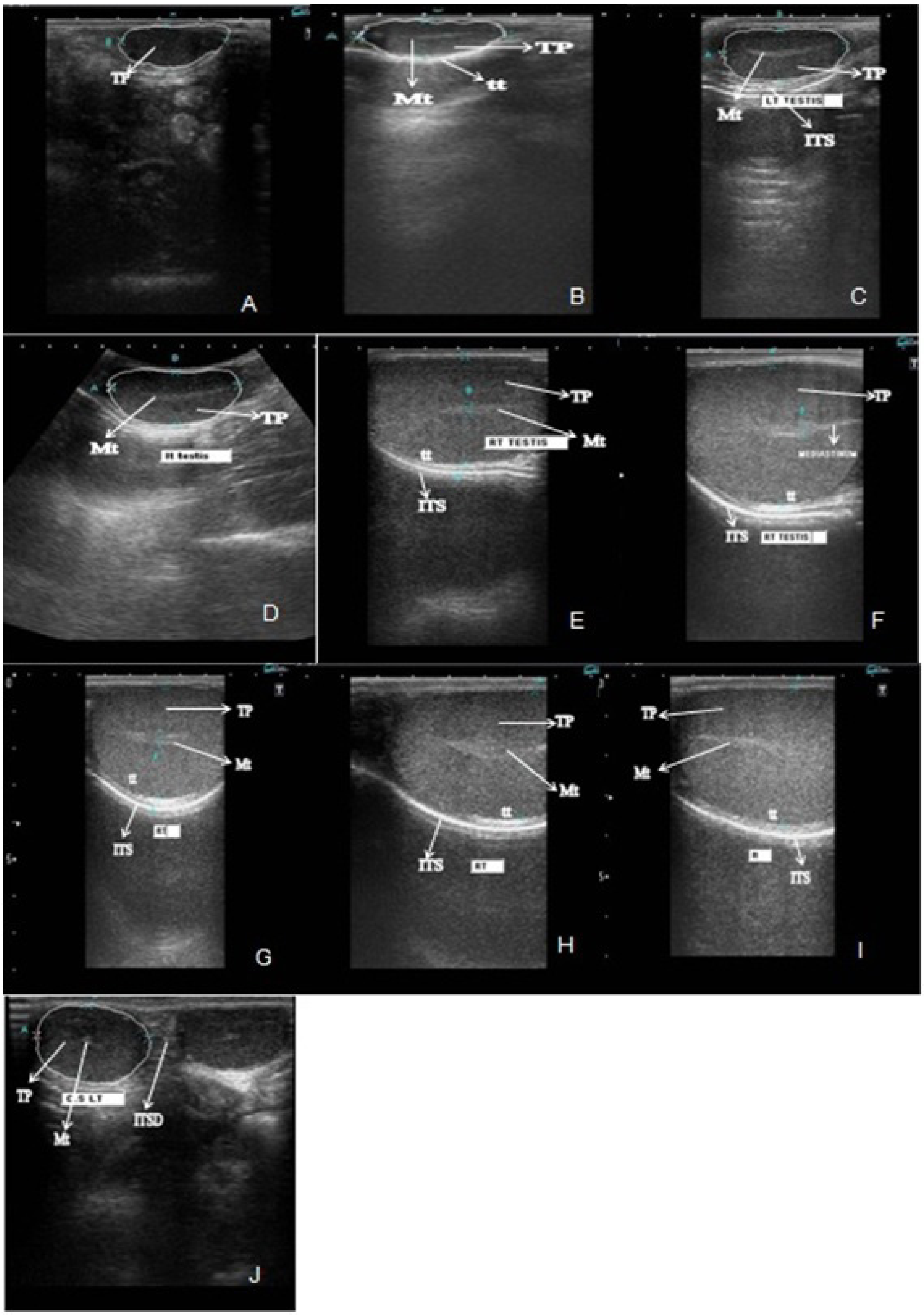

Figure 1. Ultrasound images of testes from 1 to 9 months of age using 6.0 MHz linear transducer. Longitudinal images of testis between 1 to 9 months of age (A – I, respectively) in buck and cross-section image of testis (J) at 3 months of age. (Mt: mediastinum testis, TP: testicular parenchyma, ITS: Inter Testicular Septum, ITSD: Inter Testicular Septal Depression, TT: testicular tunics).

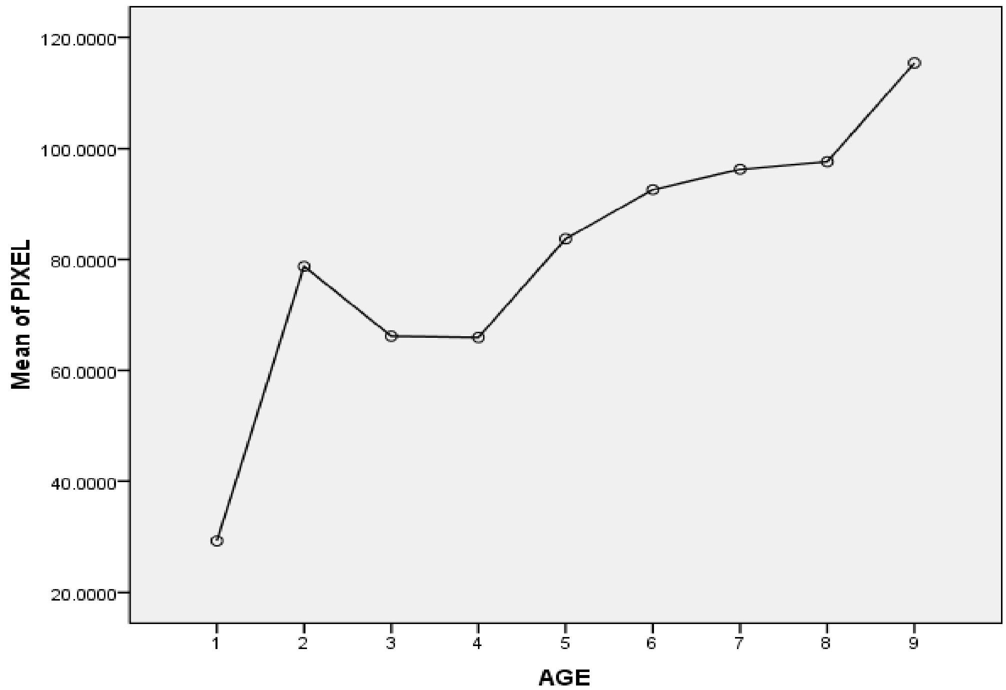

Figure 2. Showing pixel value of testes from 1 to 9 months of age.

Statistical analysis

Differences at a P-value less than 5% ($P < 0.05$) were considered to be statistically significant. One way ANOVA followed Duncan Multiple Range test were performed using the SPSS (16.0) system for windows.

RESULTS

On ultrasonography the TP appeared homogenous with a coarse medium echo-pattern (Fig. 2A-I). The testicular tunic and capsule were evident as a distinct hyperechoic line encircling the testicular parenchyma (Fig. 1). Inter testicular septum or septum scrotii appeared as a hyperechoic band between the testes on longitudinal scanning (Fig. 1) where as the inter-testicular septal depression on transverse scanning appeared anechoic thick linear band (Fig.1J). In longitudinal plane the mediastinum testis was seen as a linear structure (Fig. 1B-I) of greater echogenicity than the TP with increase in width with advancement of age and appeared as an echogenic “spot” in the centre of parenchyma in transverse plane (Fig. 1J).

The length, width and circumference of both testis increased linearly up to three months of age and then there was a steep increase in all the measurements up to seven months and slight increase in all parameters up to nine months of age. The length and circumference were measured up to 4 months of age as due to increase in size of testis the probe was not as big to cover the whole testis. With advancement of age the length, width and circumference increased. There was a significant increase ($P < 0.05$) in length and circumference of both testis from 1 to 4 months of age and similarly the width also increased significantly ($P < 0.05$) from 1 to 9 months of age (Table 1).

The pixel values of testes showed an incremental pattern, although it changed its trend at different points (Fig. 1). There was sharp increase in pixel values between 1st and 2nd month. Later on, it was steady between 2nd and 4th month of development. There was incremental increase between 4th and 7th month of development , marginal decrease at 8th month and then increase at 9th month of age.

DISCUSSION

The images of testis were taken with 6.0 MHz in longitudinal and transverse section. Other authors used 5–9 MHz/60mm (7.5MHz) linear-array transducer and a B-mode scanner for sonographic evaluation of testis in goat (Jeyakumar *et al.*, 2013). Earlier studies with boar (Ford and Wise, 2011) and stallion (Turgut *et al.*, 2005) similar position of scanning has been repoted. Recently, Andrade *et al.*, (2014) reported information about ultrasonic imaging of ram testes in peri-pubertal and pubertal Santa Inês lambs in frontal, saggital and transverse planes.

The TP in the bucks demonstrated homogeneity, with echogenicity ranging from low to moderate in both testis, regardless of scan plane used. The TP was anechoic at beginning of development of testis, as age advanced the TP became moderately echogenic. Increased echoic texture can relate to developing seminiferous tubules which, when occupied by a greater number of cells, reflecting a greater degree of acoustic waves and prevent their penetration, thus appearing as a higher density tissue (Ribadu and Nakao, 1999). These visual changes were supported by pixel values that showed increase in the beginning, steadiness afterwards with incremental changes at all points except 8 months of age. Chandolia *et al.* (1997) also reported changes in the intensity of pixel values of testis in sheep during the growth phase, noting that until the 8th week of life, there was a decrease in values, which subsequently increased up to 12 weeks of age and then stabilize and there were no changes between both testicles when evaluated in transverse and longitudinal planes. The reason of variation in IP values is not exactly known, but might be due to reflective changes in the testis. During the first week of life the values are low, reflecting the production of fluid above the spermatogenesis; pixel values gradually increase due to increased cell proliferation and the formation of mature cell types (Evans *et al.*, 1996). Therefore, these variations might be attributed to these changes.

Rate of growth of testis was most profound from third to fourth month of age. During this period the length and width increased about 12 mm and circumference about 36 mm. This increase was almost similar to left and right testis. After this period there was increase in these parameters but the increment was less as compared to third and fourth month.

Development of left and right testis was exactly same (Table.1) that is different than bull (Chandolia *et al.*, 1997), but similar to Ram (Chandolia *et al.*, 1997 and Andrade *et al.*, 2014). The mediastinum testis was visualised as a linear structure of greater echogenicity than the TP when viewed in the transverse plane and nearly circular echogenic spot in the midline of the testis when viewed in transverse plane, confirming previous study (Jeyakumar *et al.*, 2013).

In the present study no abnormalities were observed in the testes. Testicular abnormalities can be easily diagnosed with ultrasonography (Ahmad *et al.* 1991, Ahmad and Noakes 1995).

CONCLUSION

From current study it can be concluded that the development of left and right testis is exactly the same. In pre-pubertal period of development of bucks maximum growth of testis was seen between three and four months of age. Pixel values do not show a continuous pattern of growth as these alterations can be predictive of changes in the testis at different age of the animal. Thus, ultrasonography can be used for diagnosis of lesions of testis by comparing with the normal ultrasonogram.

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