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Age-related changes in testicular parameters and their relationship to thyroid hormones and testosterone in male Murrah buffaloes

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Abstract. The present study aims to investigate the age-related changes in testicular parameters and their association with plasma triiodothyronine (T_3), thyroxine (T_4), and testosterone in male Murrah buffaloes. Testicular measurements and single blood samples were collected from male Murrah buffaloes ($n = 103$) aged between 6 months and 8 years. The correlation coefficients of average testicular length (ATL), paired testis width (PTW), and scrotal circumference (SC) in relation to age were 0.88, 0.91, and 0.90, respectively. The regression equation between testicular weight (TW) and age was $Y = 1.48 \times x^{0.005}$ ($r = 0.90$; $R^2 = 0.79$). Plasma T_4 and testosterone increased significantly ($p < 0.001$) with age and their levels ranged between 12.9 and 41.8 and 0.05 and 1.48 ng mL^{-1}, respectively. With respect to associations between testicular parameters and plasma hormone levels, we observed significant ($p < 0.01$) correlations between ATL, PTW, SC, TW, and plasma T_4. A significant correlation ($r = 0.31$; $p < 0.01$) between plasma T_4 and testosterone levels was also observed. However, the correlations between plasma T_3 and testicular parameters and plasma T_3 and testosterone were non-significant. From the present study, we conclude that plasma T_4 is positively correlated with testicular parameters and plasma testosterone, indicating its role in testis development and steroidogenesis.

1 Introduction

Bull fertility is an important aspect because one bull may serve around 20 females under natural conditions or hundreds of thousands under an artificial insemination program (Devkota et al., 2011). Reliable fertility information can be obtained on several bulls by using them to impregnate a large number of cows or by evaluating their semen quality. However, these assessments are time consuming and require the necessary equipment and skills. The inadequacies of these techniques has compelled researchers to identify simple and reliable parameters to predict sperm output and fertility. Testicular weight is one among such parameters that provides an accurate estimate of the amount of sperm-producing parenchyma in the testis (Amann and Almquist, 1962; Coulter and Foote, 1977). In live bulls, testicular weight could be derived by indirect measurements such as testicular length and width and scrotal circumference (Bailey et al., 1998).

Thyroid hormones are critical regulators of growth, development, and metabolism in virtually all tissues, and altered thyroid status affects many organs and systems. For a long period of time, testis was regarded as an organ unresponsive to thyroid hormones (Wagner et al., 2008). However, recent studies have demonstrated its role in testicular development and function. The active thyroid hormone receptor isoforms (TRα1 and TRβ1) are present in Sertoli, Leydig, peritubular, and germ cells of testis (Buzzard, 2000; Canale et al., 2001; Rao et al., 2003). Thyroid hormone inhibits the proliferation and stimulates the differentiation of immature Sertoli cells during the post-natal period (Matta et al., 2002; Jansen et al., 2007). It also inhibits the proliferation of mesenchymal Leydig cell precursors and promotes the formation of adult Leydig cells (Teerds et al., 1998; Ariyaratne et al., 2000). Triiodothyronine increases the basal as well as LH (luteinizing hormone)-stimulated testosterone production by upregulating the enzymes involved in the conversion of cholesterol to testosterone (Maran et al., 2000; Manna et al., 2001).

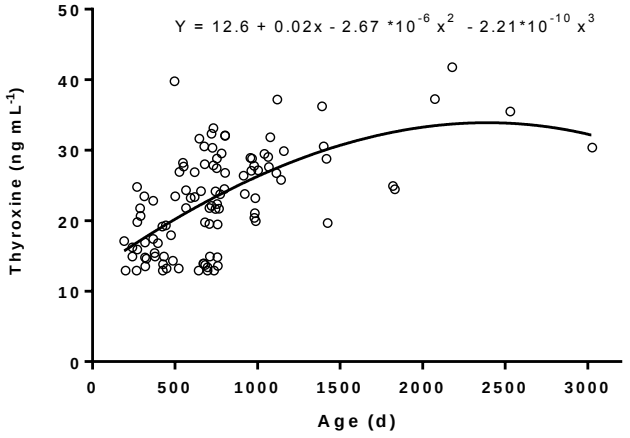

Figure 1. Age-related variations in plasma thyroxine concentrations in male Murrah buffaloes ($n = 103$).

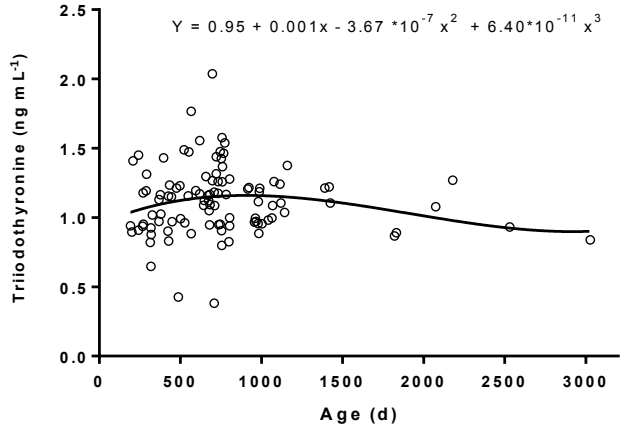

Figure 2. Age-related variations in plasma triiodothyronine concentrations in male Murrah buffaloes ($n = 103$).

Although the literature indicates the involvement of thyroid hormones in the development of testis and the production of testosterone, very few in vivo studies have been conducted to determine their association. With this perspective, the present study was designed to investigate the developmental changes in testicular parameter and its association with thyroid hormones and testosterone in male Murrah buffaloes.

2 Material and methods

2.1 Location of the study and details of environmental variables

The study was conducted at the Livestock Research Centre and the Artificial Breeding Complex of National Dairy Research Institute (ICAR-NDRI), Karnal, India. The Institute is situated at an altitude of 250 m above mean sea level, latitude and longitude being 29°42″ N and 79°54″ E, respectively. During the experimental period, the monthly average temperature and relative humidity fluctuated between 19.3 and 30.3 °C and 47 and 94 %, respectively.

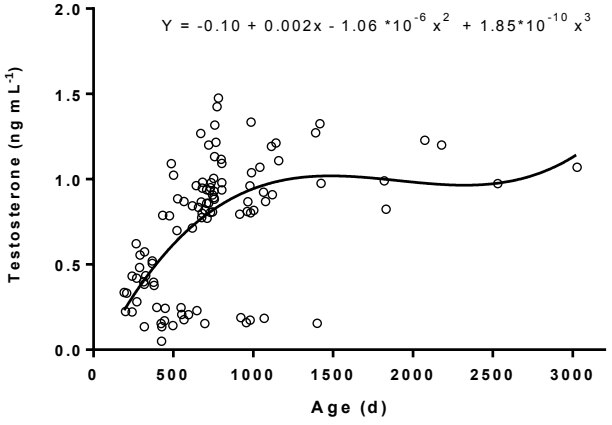

Figure 3. Age-related variations in plasma testosterone concentrations in male Murrah buffaloes ($n = 103$).

2.2 Experimental animals, blood sampling, and testicular measurements

In order to observe the age-related changes in plasma testosterone and thyroid hormones, male Murrah buffaloes ($n = 103$) aged between 6 months and 8 years were selected. Single blood sample was collected from each buffalo in sterile heparinized vacutainer tubes by jugular venepuncture. Immediately after blood collection, the samples were centrifuged at 1077 g for 15 min at 4 °C, and the plasma samples were stored at −20 °C until they were analysed for hormones. Prior to blood sampling, testicular parameters were measured for each male Murrah buffalo. Scrotal circumference (SC) of the testis was measured with a metal scrotal tape calibrated in centimetres. The length and width of each testis were measured using calipers. The weight of each testis of a pair (TW) was calculated by the formula: $TW = 0.5533(L)(W)^2$, where L is length and W is width of the testis (Bailey et al., 1998). Animal experimentation methods were approved by the Institutional Animal Ethical Committee of ICAR-National Dairy Research Institute (1705/GO/ac/13/CPCSEA Dt. 3/7/2013).

2.3 Hormone assays and statistical analysis

Plasma testosterone concentrations were determined using a bovine testosterone ELISA kit (MBS704341; MyBioSource, Inc., San Diego, California, USA). Plasma triiodothyronine (T_3; CEA453Ge) and thyroxine (T_4; CEA452Ge) concentrations were determined using multi-species ELISA kits obtained from USCN Life Science, Inc., Wuhan, China. The sensitivity levels of testosterone, T_3, and T_4 assays were 0.05, 0.047, and 1.42 ng mL^{-1}, respectively. The intra-assay

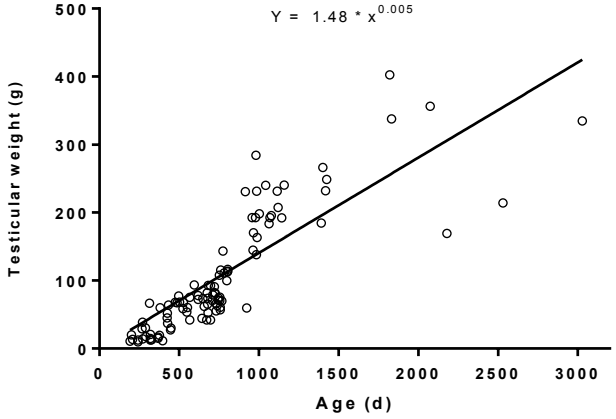

Figure 4. Age-related distribution of average testicular weight in male Murrah buffaloes ($n = 103$).

Table 1. Pearson's correlation between testosterone (T), thyroxine (T_4), triiodothyronine (T_3), average testes length (ATL), paired testes width (PTW), scrotal circumference (SC), and testicular weight (TW) in male Murrah buffaloes.

	T	T_4	T_3	ATL	PTW	SC
T_4	0.31*					
T_3	0.13	0.01				
ATL	0.45*	0.50*	−0.08			
PTW	0.51*	0.61*	−0.04	0.92*		
SC	0.49*	0.58*	−0.03	0.91*	0.96*	
TW	0.40*	0.53*	−0.10	0.91*	0.95*	0.89*

* Superscript denotes significant correlations ($p < 0.01$).

coefficients of variation for testosterone, T_3, and T_4 were 3.4, 5.6, and 4.0 %, respectively. The inter-assay coefficients of variation for testosterone, T_3, and T_4 were 5.9, 7.2, and 6.5 %, respectively. Age-related changes in plasma testosterone, T_3, T_4, and testicular parameters were analysed by different regression models. The model that gave the highest R^2 was chosen to correlate each of the parameters with age and to draw the regression trend line. Pearson's correlation was used to determine the association between thyroid hormones, testosterone, and testicular parameters. GraphPad prism (version 7) and SPPS (version 16) softwares were used for statistical analysis.

3 Results and discussion

The plasma concentrations of T_4 and T_3 and their trend lines in relation to age are represented in Figs. 1 and 2, respectively. The plasma T_3 and T_4 levels ranged between 0.38 and 2.04 and 12.9 and 41.8 ng mL^{-1}, respectively. The regression equation between T_4 concentrations and age was $Y = 12.6 + 0.02x - 2.67 \times 10^{-6}x^2 - 2.21 \times 10^{-10}x^3$ ($r = 0.61$; $R^2 = 0.37$). The regression equation between T_3 concentrations and age was $Y = 0.95 + 0.001x - 3.67 \times 10^{-7}x^2 + 6.40 \times 10^{-11}x^3$ ($r = 0.2$; $R^2 = 0.04$). The results indicated a moderate relation between T_4 concentrations and age, whereas a poor relation between T_3 concentrations and age was observed. We did not observe a definite trend line of increase or decrease in plasma T_3 levels with age, which was in agreement with a previous study conducted on Murrah buffaloes (Pandita et al., 2016).

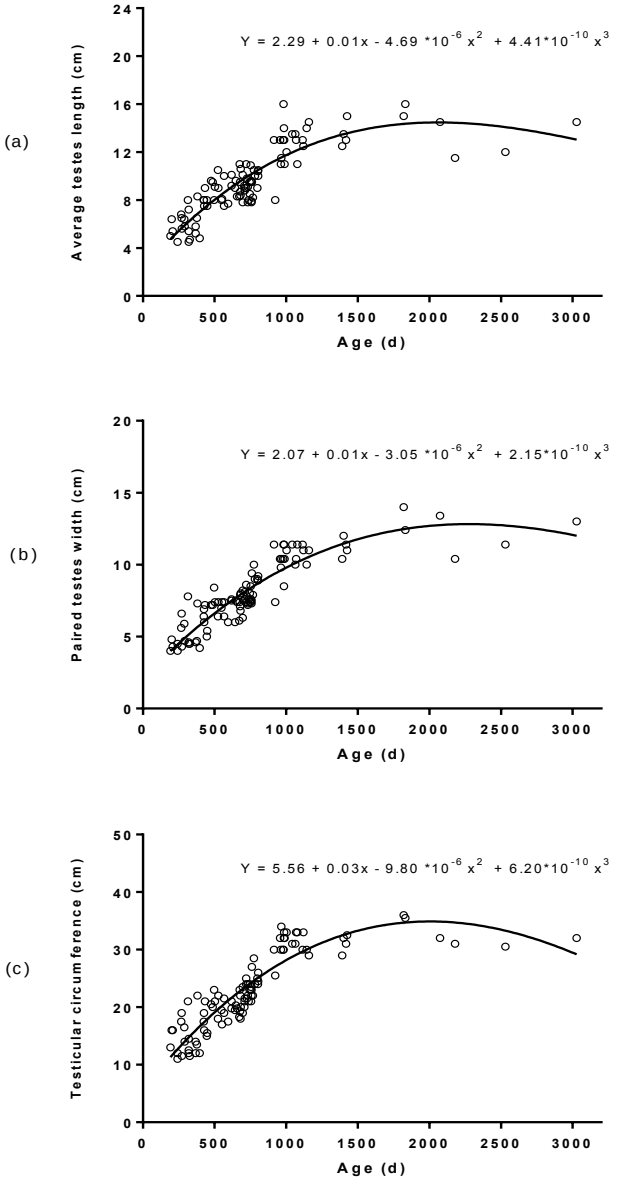

Figure 5. Age-related distribution of average testes length **(a)**, paired testes width **(b)**, and scrotal circumference **(c)** in male Murrah buffaloes ($n = 103$).

We observed a significant ($p < 0.001$) increase in plasma testosterone with age and its levels ranged between 0.05 and 1.48 ng mL^{-1} (Fig. 3). The regression equa-

tion between testosterone concentrations and age was $Y = -0.10 + 0.002x - 1.06 \times 10^{-6}x^2 + 1.85 \times 10^{-10}x^3$ ($r = 0.58$, $R^2 = 0.34$). The age-related changes in testosterone concentrations, range of testosterone, and the correlation coefficient value observed in the present study were in accordance with a similar study carried out on male Murrah buffaloes (Gulia et al., 2010). A linear relation in plasma testosterone with age was observed among the animals aged between 192 and 708 days. Similar changes in plasma testosterone were also noticed in investigations conducted on Egyptian buffalo bulls (Hemeida et al., 1985), Angus bull calves (Moura et al., 2001), Angus and Angus × Charolais bull calves (Brito et al., 2007), and Japanese Black bull calves (Kawate et al., 2011).

The average TW in relation to age ranged between 9.86 and 403 g (Fig. 4). The regression equation between average TW and age was $Y = 1.48 \times x^{0.005}$ ($r = 0.90$; $R^2 = 0.79$). The dispersion of testicular length, width, and circumference in relation to age and their regression equations are depicted in Fig. 5. The average testicular length (ATL), paired testicular width (PTW), and SC in relation to age ranged between 4.50 and 16.0, 4.00 and 14.0, and 11.0 and 36.0 cm, respectively. The correlation coefficients of ATL, PTW, and SC in relation to age were 0.88, 0.91, and 0.90, respectively. A similar positive correlation of testicular measurements and indices with age was also observed in Holstein (Coulter et al., 1975; Coulter and Foote, 1976), Angus (Coulter et al., 1975; Coulter and Keller, 1982), Hereford (Coulter and Keller, 1982; Menegassi et al., 2011), Charolias (Menegassi et al., 2011), Sahiwal (Ahmad et al., 2011), and Murrah buffalo (da Luz et al., 2013) males. The age-related distribution of testicular parameters (Fig. 5) observed in the present study agrees with the findings of a previous study (Coulter et al., 1975), which inferred that testicular size increases rapidly in young bulls and more gradually in mature bulls.

The associations between testicular parameters, plasma testosterone, T_3, and T_4 are given in Table 1. We observed significant ($p < 0.01$) correlations between plasma T_4 and testicular parameters. A significant correlation ($r = 0.31$; $p < 0.01$) between plasma T_4 and testosterone levels was also observed. However, the correlations between plasma T_3 and testicular parameters and plasma T_3 and testosterone were non-significant. Although T_3 is the bioactive form that exerts the developmental (Ariyaratne et al., 2000; Jansen et al., 2007) and steroidogenic effects (Maran et al., 2000; Manna et al., 2001) on testicular tissue, T_4 also participates directly in testicular development by promoting amino acid accumulation in Sertoli cells (Menegaz et al., 2006). Moreover, most of the T_4 is converted to T_3 in the testicular tissue. The deiodinases (D1 and D2) involved in the conversion of T_4 to T_3 are expressed in testis from weanling to adult life (Bates et al., 1999).

4 Conclusion

From the present study, we conclude that plasma T_4 levels are positively correlated with testicular parameters and plasma testosterone, indicating the developmental and steroidogenic effects of thyroid hormones on the testis.

Data availability. Data are available from the corresponding author upon request.

Author contributions. BSBK and SP designed the experiment, analysed the data, and drafted the manuscript. VGJ and BSBK performed blood sampling, measured testicular parameters, and conducted laboratory analysis.

Competing interests. The authors declare that they have no conflict of interest.

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