INTER-RELATIONSHIP OF PERIPHERAL HORMONES (IGF-1, TESTOSTERONE AND GROWTH HORMONE) WITH REPRODUCTIVE TRAITS IN MALE BUFFALO

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ABSTRACT

This study was aimed to decipher the inter-relationship peripheral hormones [Insulin-like growth hormone (IGF-1), testosterone and growth hormone] with body weight, body condition score and scrotal circumference across age-groups in male buffalo. Male buffalo (n=20) of different age groups viz. Group 1 (0 to 8 months), 2 (9 to 16 months), 3 (17 to 24 months) and 4 (25 to 32 months) were selected and Blood was collected along with body weight, body condition score and scrotal circumference. Significant difference (P<0.05) in the body weight, body condition core and scrotal circumference was observed between the groups. Peripheral IGF-1 level increased with age, highest in Group 4 (202.4±9.36 ng/ml). Similarly, testosterone was different between Group 1, 2 and 4, highest in Group 4 (1.73±0.02 ng/ml). Growth hormone, differed (P<0.05) between Group 1 (3.65±0.50 ng/ml), Group 3 (3.65±0.50 ng/ml) and Group 4 (8.56±1.96 ng/ml). Positive correlation (P<0.05) between various parameters (body weight, body condition score and scrotal circumference, testosterone and growth hormone) was observed. In conclusion, this study reports the age-related variations and inter-relationships of peripheral hormones with body weight, body condition score and scrotal circumference in male buffalo.

Keywords: Bubalus bubalis, buffaloes, male, age, IGF-1, testosterone

INTRODUCTION

Several factors influence the reproductive efficiency in cattle and buffaloes both contributed by female and male. In females, the contributing factors include parity days postpartum, uterine environment, condition of genital organs, nutrition; estrus detection and AI technique (Gokhale and Bhagat, 2000; Kumaresan and Ansari, 2001). The factors from male mainly include bull variation, sperm concentration, semen quality and quantity and freezability influenced by various hormones and growth factors including insulin-like growth Factor 1 (IGF-1) (Andersson et al., 2004; Kastelic et al., 2013).

Insulin-like growth Factor 1 (IGF-1), a protein encoded by IGF-1 gene (Hoppener et al., 1985), is primarily used by the cells as autocrine/paracrine communicator through cell surface
receptors (IGF-1R and IGF-2R), IGF-binding proteins (IGFBP-1 to IGFBP-6). IGF-1 plays an important role during pre- and post-natal growth through multiple IGFBPs. Growth hormone (GH) on its binding to its liver receptors stimulates the synthesis and release of IGF-1 peptide into circulation, where in turn binds with specific IGFBPs thereby circulates as endocrine form. IGF-1 plays an important role in different mammalian reproductive events in both male and female (Ipsa et al., 2019; Neirijnck et al., 2019). In male, IGF-1 is present in seminal plasma secreted by Leydig and Sertoli cells (Fabbrocini et al., 2000), and plays role in spermatogenesis and steroidogenesis (Lee et al., 2016). The seminal plasma IGF-1 is primarily secreted from testis and/or epididymis and its production is controlled by the gonadotropins (FSH and LH) (Lejeune et al., 1996).

With respect to peripheral IGF-1, there are several observations regarding the IGF-1 concentrations and seminal plasma concerning male fertility. In horses, greater pregnancy rates were reported when inseminated with stallion semen with higher seminal plasma IGF-1 concentrations (Macpherson et al., 2002). Likewise, abnormalities in the serum and seminal IGF-1 milieu are associated with male infertility (Cao et al., 2003; Hassan et al., 2008; Lee et al., 2016). There are no reports of serum IGF-1 concentration in bovine bulls of breeding age but in young bulls, the IGF-1 concentration ranged from 300 to 400 ng/ml with a gradual increase with age and body weight (Bourgon et al., 2017). Reports indicate that factors viz. genetics, nutrition, steroids and growth hormone concentration contribute to variations in serum and/or semen IGF-1 concentrations (Henricks et al., 1998; Macpherson et al., 2002; Selvaraju et al., 2009). In male buffalo, study showed that serum IGF-1 increasing in buffalo calves and serum IGF-1 was lower in the sub-fertile group as compared to the normal counterparts (Kumar et al., 2019), but there is no information regarding age-related changes of IGF-1 in male buffalo as well its inter-relationship with other peripheral hormones (testosterone and growth hormone), body weight, body condition score and scrotal circumference in male buffalo. Considering this, the present study was planned with the hypothesis to test any age-related variations as well as the inter-relationships of peripheral hormones (IGF-1, testosterone and growth hormone) with body weight, body condition score and scrotal circumference in male buffalo.

MATERIALS AND METHODS

Location of study
This investigation work was carried out in Semen Freezing Laboratory, ICAR-Central Institute for Research on Buffaloes, Hisar located at 29.17° N latitude and 75.72° E longitude, 212 metres above mean sea level. All experimental procedures were carried out as per guidelines of Institutional Animal Ethics Committee (IAEC) of ICAR-Central Institute for Research on Buffaloes, Hisar, India.

Study animals and measurement of body weight and body condition score and scrotal circumference
Male buffalo (n=80) in different Age groups viz. Group 1 (n=20; 0 to 8 months), Group 2 (n=20; 9 to 16 months), Group 3 (n=20; 17 to 24 months), Group 4 (n=20; 25 to 32 months) were selected for this study. Based on the farm records, the average age of the animals selected were normally distributed under different groups as 4±1, 12±1, 20±1 and 28±1 months in Group 1, 2, 3
and 4, respectively and this study was conducted from June to July during the year 2019. All study animals were maintained under uniform semi-intensive housing with feeding regime as per Nutrient Requirement of Cattle and Buffalo (2013).

Measurement of body weight and body condition score

The body weight (kg) of animals was measured using an electronic weighing scale mounted on the metallic platform at the time of blood collection. BCS was estimated on a scale of 1 to 5 with an increment of 0.25 (Edmonson et al., 1989; Anitha et al., 2010).

Measurement of scrotal circumference

Scrotal circumference (cm) of the study animals were measured using a flexible tape as reported earlier (Pawan et al., 2010).

Blood collection and estimation of peripheral hormones (IGF-1, testosterone and growth hormone)

Blood samples (10 ml) were collected at 7.00 am the morning (before morning feeding and watering) via jugular venupuncture in serum separator tubes containing clot activator. After gentle mixing, blood was allowed to clot for one hour at room temperature, followed by centrifugation at 2500 rpm for 15 minutes. Using clean pipette technique 500 µl aliquot of serum was transferred into labelled duplicate cryo-vials. Immediately after transfer vials were kept in a deep freezer at -80°C until hormone estimation. Commercially available ELISA kits were used for the estimation of serum IGF-1 (Cat. No. SEA050Bo, USCN Life Science Inc.), testosterone (Cat. No. K209, XEMA Co. Ltd) and growth hormone (Cat. No. K204, XEMA Co. Ltd) concentrations as per instructions. Sensitivity of the assay used were 0.63, 0.087 and 0.095 ng/ml for IGF-1, testosterone and growth hormone, respectively. The intra assay and inter-assay coefficient of variation of the assays were <10% and <12% respectively.

Statistical analyses

Data were evaluated for a normal distribution using the Shapiro-Wilk test and the homogeneity of variance using the Levene's test. If the data were not normally distributed, there was an arcsine transformation of data performed before analysis. Data were analysed by one-way ANOVA followed by Tukey’s post-hoc test. Correlations and regression among parameters (bodyweight, BCS, scrotal circumference, IGF-1, growth hormone and testosterone) were done using the Pearson’s correlation coefficient method. Regression between age and other parameters under study viz. bodyweight, BCS, scrotal circumference, IGF-1, growth hormone and testosterone were also carried out. Values with P<0.05 were considered statistically significant.

RESULTS

Bodyweight, body condition score and scrotal circumference in study groups

Bodyweight, body condition score and scrotal circumference observed in different study groups showed a significant difference (P<0.05) between the study groups clearly indicating there was an increase (P<0.05) in body weight, body condition score and scrotal circumference across Age groups (Table 1).
Peripheral IGF-1, testosterone and growth hormone in different study groups

Serum hormones (IGF-1, testosterone and growth hormone) levels deduced in different study groups is shown in Table 2. It was observed that IGF-1 level increased with age showing significant differences (P<0.05) between the groups, highest in group 4 (202.4±9.36 ng/ml). Similarly, testosterone showed difference between Group 1, 2 and 4, highest in Group 4 (1.73± 0.02 ng/ml). With respect to growth hormone, significant difference (P<0.05) was observed between Group 1, 3 and 4 (Table 2).

Inter-relationship with peripheral hormones (IGF-1, Testosterone and Growth hormone) with study parameters (Body weight, body condition score and scrotal circumference)

Correlation between various parameters (body weight, body condition score and scrotal circumference, IGF-1, testosterone and growth hormone) under study showed positive that body weight was significantly correlated (P<0.05) with body condition score (r=0.59), scrotal circumference, IGF-1 (r=0.43), testosterone (r=0.43), growth hormone (r=0.47) and age (r=0.85). Similarly, body condition score and scrotal circumference were positively correlated with all parameters under study. With respect to hormone, IGF-1 was correlated with body weight (r=0.43), scrotal circumference (r=0.43), testosterone (r=0.38) and age (r=0.45). Likewise, testosterone was correlated with all the parameters under study, and growth hormone showed correlation with all parameters, except IGF-1 (Table 3). Regression analysis of the parameters under study showed significant (P<0.05) prediction of bodyweight (R²=73), body condition score (R²=19), scrotal circumference (R²=67), testosterone (R²=19) and growth hormone (R²=24) with age (Table 4).

DISCUSSIONS

This study reports the age-related variations as well as the inter-relationships of peripheral hormones (IGF-1, testosterone and growth hormone) with body weight, body condition score and scrotal circumference in male buffalo. Selection of males is a pre-requisite to enhance the economic potential of the progenies through the breeding program, including buffaloes (Devkota et al., 2011). Investigation in buffaloes of varied age viz. ranging from ≤18 months to ≥24 months), scrotal circumference and weight varied significantly with a positive relationship with plasma testosterone. In addition, testosterone concentration showed an increasing trend with age (Mahmood et al., 2018) which agreed with our study. Study in riverine and Swamp buffalo males showed that scrotal circumferences coincided with body weight and size of scrotal circumference showed a positive correlation (Bongso et al., 1984; Singh et al., 2010; Korejo et al., 2019) being in agreement to this investigation. Likewise, age-related changes in testicular parameters and their relationship to thyroid hormones and testosterone in riverine buffaloes with positive correlation scrotal circumference with age confirming the complementary effect of these factors towards growth (Gulia et al., 2010; Jadhav et al., 2018).

With respect to hormones, the findings of testosterone levels agreed with earlier studies in adult buffalo bulls, with values ranging from 0.2 to 2.7 ng/ml with peripheral testosterone concentrations were lower in younger bulls (Gunarajasingam et al., 1985). Likewise, study in cattle (Kawate et al., 2011) and buffalo (Jadhav et al., 2018) males showed that plasma thyroxine and testosterone increased significantly with age. In cattle and buffalo, increase in serum testosterone
between with age, followed by testicular and body growth is corroborated in this study considering the synergistic action of these hormones towards growth (Kastelic et al., 2013; Sakase et al., 2018; Ipsa et al., 2019). With respect to IGF-1, our findings of peripheral IGF-1 was in consonance with reports (Lee et al., 2005; Byrne et al., 2018); wherein the peripheral IGF-1 levels reached from +50 to 200 ng/ml with the onset of puberty in bulls. Moreover, significant correlation observed between IGF-1 and testosterone concentrations indicate a close relationship between peripheral IGF-1 and testicular development, for stimulating the body as well as testicular development due to their mitogenic effect (Davis et al., 2002; Byrne et al., 2018; Sakase et al., 2018). The change in live weight was found to be positively correlated to the plasma IGF-1 concentration \((r=0.68)\) as plasma IGF-1 concentration gradually increased with age in cattle (Lee et al., 2005; Byrne et al., 2017). In Holstein steers, a strong correlation was observed between age and IGF-1 plasma concentration (Torrentera et al., 2009) and significant effect of age on body weight was observed in Japanese Black beef bulls has been reported (Sakase et al., 2018). Similar to our results on peripheral IGF-1 and testosterone, there was an increase in GH with age as reported earlier (Kerr et al., 1991; Renaville et al., 1993; 1996; 2000). Regarding scrotal circumference, significant effects of age and on the bulls’ plasma IGF-1 concentrations was observed with lower plasma IGF-1 concentrations in abnormal bulls (Weerakoon et al., 2018) which was in contrast with our findings in buffalo bulls (Kumar et al., 2019) attributing to species difference and possible mechanism to be elucidated in future. Likewise, in Japanese Black beef bull calves, significant effects of age on the body weight and scrotal circumference was observed and plasma testosterone and IGF-1 concentrations were positively correlated with scrotal circumference (Sakase et al., 2018), as observed in in buffalo counterparts.

In rams, dietary energy had an effect on seminal plasma insulin-like growth factor-1 (IGF-I), serum IGF-1 and testosterone levels, semen quality and fertility in adult males (Selvaraju et al., 2012). Similarly in Andalusian stallions, serum IGF-1 concentrations determined in different sex and Age groups (Munoz et al., 2011) were in agreement with our findings. From this study, it can be evident that scrotal circumference in Bos taurus, Bos indicus and Bubalus bubalis varied with body weight and age and the inter-relationship of the study parameters (body weight, BCS, scrotal circumference, testosterone and growth hormone and IGF-1) agrees with reports in cattle and buffalo (Lee et al., 2005; Torrentera et al., 2009; Singh et al., 2010; Brito et al., 2012; Byrne et al., 2018; Jadhav et al., 2018). These factors inter-play for the onset of reproductive functions and periodic observation of these parameters may act as cue as well intervention yardsticks for enhancing the puberty and sexual maturity in male buffalo.

In conclusion, this is the first study to report the inter-relationships of peripheral hormones (IGF-1, testosterone and growth hormone) with body weight, body condition score and scrotal circumference across age groups in male buffalo.

ACKNOWLEDGEMENTS

The authors thank the Director, ICAR-Central Institute for Research on Buffaloes as well as for providing necessary facilities for conducting this study. The authors thank Animal farm section, ICAR-CIRB and LUVAS, Hisar
Table 1. Body weight, body condition score and scrotal circumference in different study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (kg) (n = 20)</th>
<th>Body condition score (1-5) (n = 20)</th>
<th>Scrotal circumference (cm) (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96.26±6.62^A</td>
<td>2.67±0.05^A</td>
<td>12.89±0.51^A</td>
</tr>
<tr>
<td>2</td>
<td>178.4±9.54^B</td>
<td>2.71±0.04^A</td>
<td>17.25±0.49^B</td>
</tr>
<tr>
<td>3</td>
<td>265.5±9.35^C</td>
<td>2.88±0.04^B</td>
<td>20.9±0.94^C</td>
</tr>
<tr>
<td>4</td>
<td>368.2±4.42^D</td>
<td>2.94±0.04^C</td>
<td>25±0.58^D</td>
</tr>
</tbody>
</table>

^A-D Values expressed as Mean ± SEM; Values with different superscript in a column differ significantly (P<0.05).

Table 2. Hormones (IGF-1, testosterone and growth hormone) levels in different study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IGF-1 (ng/ml) (n = 20)</th>
<th>Testosterone (ng/ml) (n = 20)</th>
<th>Growth hormone (ng/ml) (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100±9.05^A</td>
<td>1.52±0.02^A</td>
<td>3.65±0.50^A</td>
</tr>
<tr>
<td>2</td>
<td>130.6±9.13^A</td>
<td>1.62±0.05^AB</td>
<td>6.83±1.26^AB</td>
</tr>
<tr>
<td>3</td>
<td>188.1±8.16^B</td>
<td>1.66±0.03^B</td>
<td>8.63±1.8^B</td>
</tr>
<tr>
<td>4</td>
<td>202.4±9.36^C</td>
<td>1.73±0.02^C</td>
<td>8.56±1.96^B</td>
</tr>
</tbody>
</table>

^A-C Values expressed as Mean ± SEM; Values with different superscript in a column differ significantly (P<0.05).

Table 3. Correlation between various parameters under study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BW</th>
<th>BCS</th>
<th>SC</th>
<th>IGF-1</th>
<th>T</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCS</td>
<td>0.59**</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>0.94**</td>
<td>0.52**</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1</td>
<td>0.43**</td>
<td>0.20</td>
<td>0.43**</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.43**</td>
<td>0.22*</td>
<td>0.46**</td>
<td>0.38**</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>0.47**</td>
<td>0.31*</td>
<td>0.52**</td>
<td>0.24</td>
<td>0.30*</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.85**</td>
<td>0.46**</td>
<td>0.82**</td>
<td>0.45**</td>
<td>0.42**</td>
<td>0.35**</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; BW: Body weight; BCS: Body condition score; SC: Scrotal circumference; IGF-1: Insulin-like growth hormone; T: Testosterone; GH: Growth hormone.
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**REFERENCES**


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Table 4. Prediction equations for the BW, BCS, SC, IGF-1, testosterone and GH on the basis of age.

<table>
<thead>
<tr>
<th>Predicted equations</th>
<th>$R^2$ (%)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\hat{Y} (BW)$</td>
<td>73</td>
<td>68.75**</td>
</tr>
<tr>
<td>$\hat{Y} (BCS)$</td>
<td>19</td>
<td>5.81**</td>
</tr>
<tr>
<td>$\hat{Y} (SC)$</td>
<td>67</td>
<td>52.25**</td>
</tr>
<tr>
<td>$\hat{Y} (IGF1)$</td>
<td>5</td>
<td>1.20</td>
</tr>
<tr>
<td>$\hat{Y} (Testosterone)$</td>
<td>19</td>
<td>5.61**</td>
</tr>
<tr>
<td>$\hat{Y} (GH)$</td>
<td>24</td>
<td>3.19*</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; BW: Body weight; BCS: Body condition score; SC: Scrotal circumference; IGF-1: Insulin-like growth hormone; GH: Growth hormone.


Gulia, S., M. Sarkar, V. Kumar, H.H.D. Meyer and B.S. Prakash. 2010. Divergent development of testosterone secretion in male zebu (Bos indicus) and crossbred cattle (Bos indicus x Bos taurus) and buffaloes (Bubalus bubalis) during growth. Trop. Anim. Health Pro., 42(6): 1143-1148. DOI: 10.1007/s11250-010-9538-x


peroxidation and fructose uptake in vitro. 