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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 circumfermnce 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). Postive 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 inflcuend 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

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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 circumfermnce 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 circumfermnce 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^{o}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 co-efficient 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 diffence ($P<0.05$) 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 ($R^2=73$), body condition score ($R^2=19$), scrotal circumference ($R^2=67$), testosterone ($R^2=19$) and growth hormone ($R^2=24$) 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). Investiagtion 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 age 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 comfirming the complementray 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 studie 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 tthat 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.

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Table 1. Body weight, body condition score and scrotal circumference in different study groups.

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

$^{A-D}$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.

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

$^{A-C}$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.

Parameters	BW	BCS	SC	IGF-1	T	GH
BW	--	--	--	--	--	--
BCS	0.59**	--	--	--	--	--
SC	0.94**	0.52**	--	--	--	--
IGF-1	0.43**	0.20	0.43**	--	--	--
T	0.43**	0.22*	0.46**	0.38**	--	--
GH	0.47**	0.31*	0.52**	0.24	0.30*	--
Age	0.85**	0.46**	0.82**	0.45**	0.42**	0.35**

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

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

Predicted equations	R^2 (%)	F-value
Yˆ(BW) = 47.64 + 12.34 (Age) + -0.14 (Age^2) + 0.003 (Age^3)	73	68.75**
Yˆ(BCS) = 2.82 + -0.08 (Age) + 0.001 (Age^2) + 0.00 (Age^3)	19	5.81**
Yˆ(SC) = 9.38 + 0.96 (Age) + -0.03 (Age^2) + 0.001 (Age^3)	67	52.25**
Yˆ(IGF1) = 168.70 + -15.33 (Age) + 1.16 (Age^2) + -0.002 (Age^3)	5	1.20
Yˆ(Testosterone) = 1.45 + 0.018 (Age) + 0.001 (Age^2) + -1.245 (Age^3)	19	5.61**
Yˆ(GH) = 1.05 + 0.687 (Age) + -0.01 (Age^2) + 0.001 (Age^3)	24	3.19*

*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.

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