OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

The Relationship Between Different Life Stages and the Concentration of Some Hormones in the Blood of Egyptian Buffalo Males

Hamdy Abdala Elnagar, Wael Mohamed Wafa*, Abdelaziz Mustafa Sakr, Mohammed Mahmoud Hegazy

Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Nadi El Said, Dokki, Giza 12618, Egypt.

Abstract | The current study aimed to illustrate the changes in the levels of testosterone (TES), leptin, and prolactin (PRL) in the blood and their relationships with age advancing of Egyptian buffalo males from one up to 24 month of age. Blood samples were taken from 66 male buffalo calves at 1, 3, 6, 12, 18 and 24 month of age (11 animals at every age). TES, leptin and PRL levels were determined in blood plasma. Results showed insignificant increase in TES and PRL by age advancing at 1-3 month, and in leptin at 1-6 month of age. All hormones markedly increased up to 24 month of age. TES increased by increasing body weight (BW) from 40 to 420 kg. Leptin and PRL increased by increasing BW (>200 and >120 kg, respectively). The polynomial regression equation for all hormones gave the highest R^2 values 0.889 and 0.971 for the unique measurement of age-TES and both age-leptin and age-PRL, respectively. A negative correlation was recorded between TES-leptin at all ages (r ranged between -0.656 and -0.995). The correlation between TES-PRL was significant at 1 (r=-0.701), 12 (r=-0.609), and 24 (r=-0.724) month of age. Leptin significantly correlated with PRL at 1 month (r=0.666), 6 (r= -0.659), and 24 (r=-0.804) month of age. The present results may suggest an increase in all hormonal levels (TES, leptin, and PRL), depending on progressing age or body weight of males. More acceleration was observed in testosterone than in prolactin and leptin by advancing age and/or weight of buffalo-male bulls.

Keywords | Buffalo-males, Blood, Testosterone, Leptin, Prolactin.

Received | March 28, 2022; **Accepted** | April 30, 2022; **Published** | June 20, 2022
***Correspondence** | Wael Mohamed Wafa, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Nadi El Said, Dokki, Giza 12618, Egypt; **Email:** drwaelfatoh@hotmail.com
Citation | Elnagar HA, Wafa WM, Sakr AM, Hegazy MM (2022). The relationship between different life stages and the concentration of some hormones in the blood of Egyptian buffalo males. Adv. Anim. Vet. Sci. 10(7): 1542-1550.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.7.1542.1550
ISSN (Online) | 2307-8316

INTRODUCTION

The water buffalo represents an important part of animal production in Egypt. It is economically a very important farm animal and genetic improvement of these animals is of economic importance, especially, in reproductive performance and quantity of meat and milk (Habeeb et al., 2016). The national production of buffalo milk and meat represented 66 and 43% of the national production and buffaloes had significantly higher longevity than cattle, providing calves and milk until up to 20 years of age (FAO, 2017).

The hormones had an important role in regulating growth and maintaining the basal rate of metabolism (Ingole et al., 2012). In this concern, testosterone (TES) hormone, at central and peripheral levels, regulates sexual properties of males (Vignozzi et al., 2005). In hypogonadal men, TES administration improved the sexual function (Schultheiss et al., 2000) due to its activities on spermatogenesis, repro-

ductive tract, epididymal function (sperm maturation and survival) (Goeritz et al., 2003). Levels of TES are benefits in young sire selection. Also, TES is the first responsible for puberty incidence and spermatogenesis (Goeritz et al., 2003). In Egyptian male buffalo calves from birth to 24 months of age, the changes in TES profile showed marked changes according to age and body weight advancing (Habeeb et al., 2016).

Leptin, the hormone product of the *ob* gene, is secreted mainly from adipocytes (Zhang et al., 1994). It decreases feed consumption to regulate energy balance and increases expenditure of energy (Bray and York, 1997). Leptin was affected negatively by TES levels in men (Blum et al., 1997). The reversible relationship between leptin and TES was reported by several authors (Sánchez-Garrido and Tena-Sempere, 2013; Chou and Mantzoros, 2014; Mulhall et al., 2018). In human, leptin regulates the sexual maturity and modulates neuro-endocrine systems (Wauters et al., 2000; Kawamura et al., 2002), indicating the direct control of leptin on reproductive function (Aquila et al., 2005). Also, leptin has important roles on regulating the function of gonads in males, directly via peripheral tissue membrane receptors (Tena-Sempere et al., 2007) and indirectly through the central neuro-endocrine system (Steinman et al., 2001). Sperm cells can secrete leptin to modulate sperm metabolism, in relation to energy requirements (Aquila et al., 2005).

Prolactin (PRL) is a single-chain protein containing 199 amino acids and produced in the lactotropes of the anterior pituitary gland (Nett, 1993). It has a wide range of biological actions in various species and is primarily regulated by dopamine secreted from the hypothalamus (Hadley, 2000). In females, PRL is related to pregnancy and lactation. In males, studies showed relation between sperm motility with serum PRL (Hernández Uribe et al., 2001; Pasqualotto et al., 2005). However, normal serum concentrations of PRL have been shown to exert permissive roles in the male reproductive tract (Gonzales et al., 1989), but excessive serum PRL concentration was correlated with infertility, hypogonadism, impotence, and galactorrhea (Segal et al., 1979). In the ejaculate of men, PRL stimulates utilization of glucose and fructose, activity of adenylcyclase and ATP-ase, thereby affecting the motility and fertilizbility of sperm cells. Hyperprolactinemia in men causes a lipido loss (Hafez and Hafez, 2000).

Male fertility depends on the proper function of a complex system of organs and local balance between androgen and estrogen which is important for spermatogenesis (Luboshitzky et al., 2002). Several factors such as sex, age, and other physiological states were reported to affect blood hormonal profiles of animals (Garg et al., 2002). Unfortunately, little information is available on the changes in hormonal profiles of Egyptian buffaloes with age advancing. Also, the relationships among TES, leptin, and PRL at different ages are not studied in Egyptian buffaloes. Therefore, the aim of this study was to illustrate the changes in the levels of TES, leptin, and PRL in the blood and their relationships with age advancing of Egyptian buffalo males from one up to 24 month of age.

MATERIALS AND METHODS

The experimental work was conducted at Mahlt Mousa Animal Production Station, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Egypt.

This experiment was conducted in accordance with the Directive 2010/63/EU for animal protection that used for scientific purposes (Official Journal of the European Union, 2010). All effort has been made to reduce animal suffering.

Animals

The experimental work was conducted on 66 male buffalo-calves at different ages from one until 24 months of age. Animals at 1, 3, 6, 12, 18 and 24 months of age (11 animals at every age) were chosen in this study.

Blood samples

By using disposable syringes, blood samples were withdrawn from the Jugular vein of each animal before the morning feeding (7 a.m.). Each blood sample (10 ml) was collected in test tubes containing anti-coagulant (disodium-EDTA), and all samples were transported in ice-box to the laboratory. Blood samples were centrifuged (4000 rpm for 15 min.) to separate blood plasma, which were stored at -20°C until the hormonal assay. Plasma TES, leptin, and PRL were measured by commercial kits.

Hormonal assays

Plasma PRL and TES levels were determined by the chemiluminescence method using an automated immunoassay analyzer (Immulite analyzer) (Siemens Healthcare Diagnostics, Inc., USA). Leptin concentrations in plasma were measured using a solid-phase sandwich ELISA (EAI-2395, DRG Instruments GmbH, Germany).

Statistical analysis

Data were statistically analyzed by using IBM SPSS statistical program (2017) to test the differences among ages for each hormone, then the significant differences were separated by Duncan's Multiple range test (Duncan, 1955). Partial correlation coefficients within SPSS program were used. Trend line for each hormone with age progress was estimated and regression coefficients were recorded.

Table 1: Effect of age on profiles of testosterone, leptin, and prolactin in blood plasma of male buffaloes.

Item	Concentration (ng/ml)		
	Testosterone	Leptin	Prolactin
Age 1 (1 mo)	0.05±0.01^{e}	1.07±0.03^{d}	2.47±0.11^{e}
Age 2 (3 mo)	0.27±0.03^{e}	1.09±0.02^{d}	3.28±0.16^{e}
Age 3 (6 mo)	1.06±0.18^{d}	1.11±0.03^{d}	4.95±0.25^{d}
Age 4 (12 mo)	1.53±0.11^{c}	1.41±0.03^{c}	8.98±0.40^{c}
Age 5 (18 mo)	2.10±0.14^{b}	2.05±0.02^{b}	13.83±0.42^{b}
Age 6 (24 mo)	2.40±0.11^{a}	2.72±0.04^{a}	21.50±0.58^{a}

a, b …….d: Significant differences at P<0.05 for different litters in the same column.

Table 2: Effect of live body weight categories on profiles of testosterone, leptin, and prolactin in blood plasma of male buffaloes.

Category of LBW (kg)	Concentration (ng/ml)		
	Testosterone	Leptin	Prolactin
40-60	0.05±0.01^{f}	1.07±0.03^{d}	2.47±0.11^{e}
90-120	0.56±0.13^{e}	1.08±0.02^{d}	3.76±0.25^{e}
125-200	1.19±0.09^{d}	1.29±0.05^{d}	7.47±0.77^{d}
210-300	1.68±0.11^{c}	1.73±0.13^{c}	10.65±0.99^{c}
>300-350	2.26±0.09^{b}	2.35±0.14^{b}	17.42±1.38^{b}
365-420	2.61±0.08^{a}	2.64±0.04^{a}	21.04±0.81^{a}

a, b …….d: Significant differences at P<0.05 for different litters in the same column.

Table 3: Correlation coefficients of hormonal profiles in blood serum of male buffaloes at different ages.

Item	Correlation coefficient between		
	Testosterone/Leptin	Testosterone/Prolactin	Leptin/Prolactin
Age 1 (1 mo)	-0.970**	-0.701*	0.666*
Age 2 (3 mo)	-0.656*	0.077	0.231
Age 3 (6 mo)	-0.446	-0.209	-0.659*
Age 4 (12 mo)	-0.995**	-0.609*	0.579
Age 5 (18 mo)	-0.955**	0.394	-0.504
Age 6 (24 mo)	-0.990**	-0.724*	-0.804**

* Significant at P<0.05. ** Significant at P<0.01.

RESULTS

Effect of age

Results cleared change (P<0.05) in all hormonal levels of TES, leptin, and PRL (Table 1). There was insignificant increase in TES and PRL levels by advancing age from one to three months, and in leptin level from one to 6 month of age. Then, marked and gradual increase (P<0.05) was observed in all hormones by advancing age.

Effect of live body weight category

Results showed change (P<0.05) in TES, leptin, and PRL by increasing live body weight (Table 2). Concentration of TES showed gradual and continued increase (P<0.05) by increasing body weight from 40 to 420 kg, while the increase in leptin and PRL was recorded by increasing body weight above 200 and 120 kg, respectively.

Rate of change in hormonal level with age advancing

Results illustrated in Figure 1 showed significantly (P<0.05) different trend of increasing rate for all hormones at different age intervals. The highest increasing rate in TES, PRL, and leptin was observed at 1-3, 6-12, and 12-18 month of age intervals, respectively. The lowest values of increasing rate were at 1-3 month of age for all hormones.

Trend line of hormonal profile changes

The polynomial, exponential, linear and logarithmic functions were derived to explain age and each of TES, leptin, and PRL relationship in males (Figures 2-4). The polynomial regression equation gave the highest R^2 values (0.889)

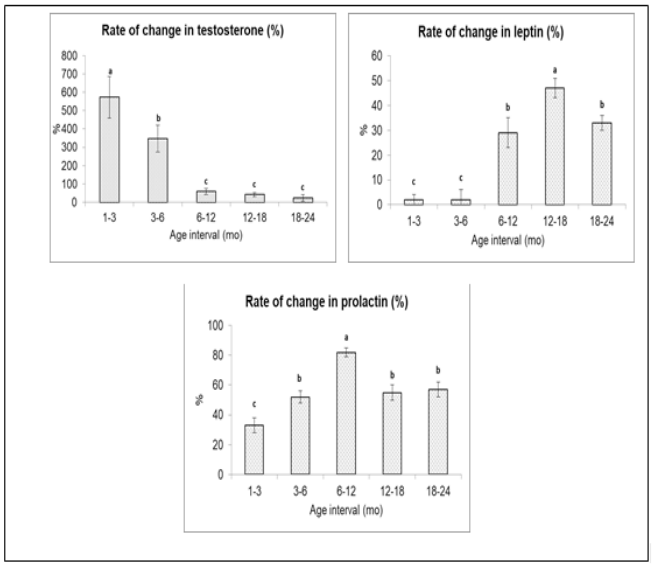

Figure 1: Increasing rate of testosterone, leptin, and prolactin levels in blood plasma of male buffaloes at different age intervals.

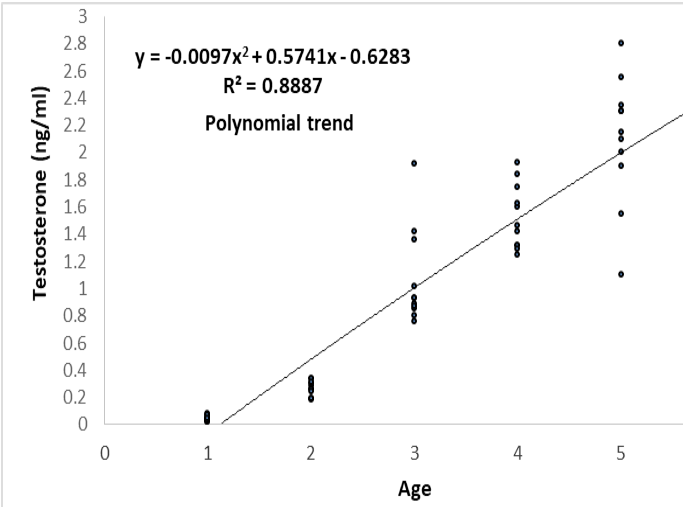

Figure 2: Trend line of testosterone profile in blood serum of male buffaloes at different ages.

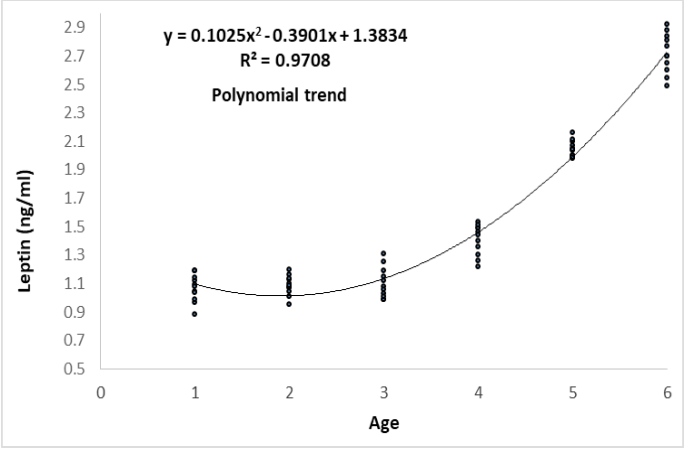

Figure 3: Trend line of leptin profile in blood serum of male buffaloes at different ages.

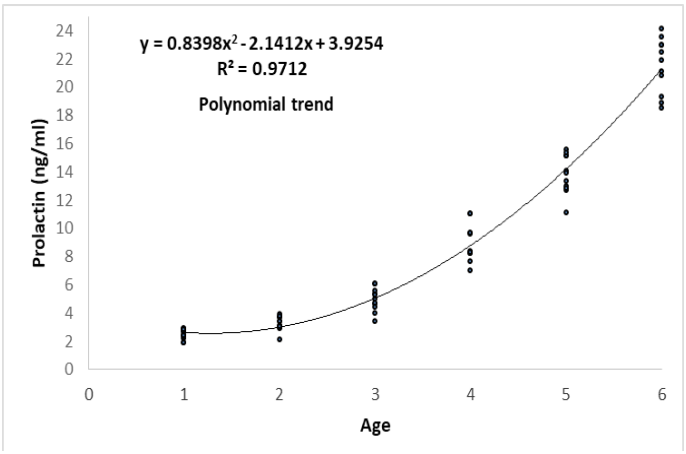

Figure 4: Trend line of prolactin profile in blood serum of male buffaloes at different ages.

for the unique measurement of age-TES. The analysis and curve fitting revealed that the polynomial function explained TES in male for ages to the extent of 88.9%, hence the equation ($y= -0.0097x^2 + 0.5741x - 0.6283$) can reliably be utilized for the prediction of TES in males, where y= TES (ng/ml) and x = age (month) (Figure 2).

The polynomial regression equation gave the highest R^2 values (0.971) for the unique measurement of age-leptin. The analysis and curve fitting showed that the polynomial function explained leptin in male for ages to the extent of 97.1%, and the equation ($y = 0.1025x^2 - 0.3901x + 1.3834$) can reliably be utilized for the prediction of leptin of males, where y= leptin (ng/ml) and x = age (month) (Figure 3).

In the same pattern, a polynomial regression equation gave the highest R^2 values (0.971) for the unique measurement of age-PRL. The analysis and curve fitting cleared that the polynomial function explained PRL in male for ages to the extent of 97.1%, and the equation ($y = 0.8398x^2 - 2.1412x + 3.9254$) can reliably be utilized for the prediction of PRL of males, where y= PRL (ng/ml) and x = age (month).

Correlation coefficients at different ages

Regarding the correlation between hormones within each age, results revealed negative correlation between TES and leptin at all ages. The correlation between TES and leptin was negatively the highest at 12 and 24 month of age (r=-0.995 and -0.990, $P<0.01$), while it was insignificant at 6 month of age. The correlation between TES and PRL was significant ($P<0.05$) and negative at 1, 12, and 24 month of age, being the highest at 24 month of age. However, leptin significantly correlated positively with PRL at 1 and negatively at 6 ($P<0.05$), being in a negative pattern (r= 0.804, <0.01) 24 month of age (Table 3).

DISCUSSION

The results concerning the change in hormones as affected by age or body weight category indicated gradual and sharp increase in TES with advancing age from 1 up to 24 month of age (polynomial trend, R^2 88.8%) or body weight from 40 up to 420 kg. Concentration of TES is related to age (Delgadillo and Chemineau 1992) and positively correlated with animal body weight (Bezerra et al., 2009; Habeeb et al., 2016). Concentration of TES was reported to increase by increasing age or body weight (Habeeb et al., 2016). The TES is important for the sex drive, energy, and behavior, therefore a significant alteration in TES profile may alarmed with advancing age (Al-Qarawi et al., 2004). In comparable with our results, the highest rate of increase in TES was observed at 1-3 month of age, followed by that at 3-6 month of age. On the other hand, the first significant increase in serum TES occurred at 8-9 months of age (Singh Shatab et al., 2016). In Italian Mediterranean buffalo bulls, body weight and level of plasma TES decreased at 5-21 months and increased at 25 months, showing the maximal levels at 38 month of age (Malfatti et al., 2006). In Holstein bulls, serum level of TES showed lower level in young bulls as compared to advanced ages. Level of serum TES elevated to 5.80 ng/ml at 48 month of age in comparing with 2.00 ng/ml at 12 month of age (Matsuzaki et al., 2000).

Puberty of swamp buffalo bull is likely to arrive at an earlier age (less than 24 months) as reported by Fischer and Bodhipaksha (1992). Other information indicated that the buffalo, both males and females, are sexually mature at the age of 3-4 years (Fischer and Bodhipaksha, 1992; Gordon, 1996). Level of serum TES increased ($P<0.05$) in bulls with acceptable and fair sexual behavior compared with those with poor sexual behavior, being 0.86±0.01, 0.69±0.02, and 0.29±0.02 ng/mL, respectively as observed by Swelum et al. (2017). Concentration of TES was higher ($P<0.05$) in adult buffalo bulls (2.93±0.44 ng/ml) than in male calves (1.20±0.35 ng/ml) (Singh Shatab et al., 2016). In our study we found that level of TES was less than 1 ng/ml at age ≤6 month of age reflecting pre-puberty stage of males. Meanwhile a pronounced increase in TES to be 2.10±0.14 ng/ml at 18 month of age which mean that bulls to be at puberty stage. At this age average TES concentration was above 2 ng/ml. Buffalo bulls can complete establishment of spermatogenesis at 24 month of age (Gordon, 1996). Moreover, TES concentration was higher ($P<0.05$) in adult buffalo bulls than in male calves (2.93±0.44 ng/ml versus 1.20±0.35 ng/ml) (Singh Shatab et al., 2016). They observed that in serum TES concentrations were very low (18.0 ± 2.9 pg/ml) in buffalo bull calves at birth and remained low up to 8 month of age. The obtained results revealed that plasma TES concentration was 2.40 ng/ml at 24 month of age and 2.26 for LBW category of >300-350 kg which may suggest the sexual maturity of most bulls at this age/weight.

According to our results, there was a slight increase in leptin to 12 month of age or >200 kg BW, and in PRL from 1 to 6 month of age or body weight from 40 to 120 kg. Later and marked increase in leptin and PRL (polynomial trend, R^2 = 97.1% for each) was recorded at 12 and 6 month of age, respectively. Leptin is secreted by the white adipose tissue in proportion to body energy (fat) stored. It controls the reproductive function of males (Gholami et al., 2010) and regulates animal body weight (Tena-Sempere et al., 2007; Glander et al., 2002). Many authors reported that leptin showed a reduction (Rosenbaum et al., 1996), no change (Ahrén and Pacini, 1998), or an increase (Baumgartner et al., 1999) during aging. In human, the differences in serum leptin were reported between young and old males, and age may affect leptin secretion in non-obese and obese males (Isidori et al., 2000). In addition, blood leptin decreased by 53% in older males (Ostlund et al., 1996). The central response of leptin was also impaired by aging (Pétervári et al., 2014). Down-regulation of megalin expression occurred by aging (Dietrich et al., 2008), resulting in a reduction of leptin uptake in the hypothalamus due to decreasing leptin receptor mRNA expression (Fernández-Galaz et al., 2001), and protein of leptin receptors in the hypothalamus (Scarpace et al., 2001).

The negative correlation between leptin and TES at different ages in our study was attributed to the observed increase in TES or leptin level with age progress of buffalo bulls. In this respect, *in vitro* study of Wabitsch et al. (1997) on human cleared that TES had a direct long term inhibitory effect on production of leptin from adipocytes. In men, cross-sectional study has reported that TES negatively affected leptin level in blood independently of body fatness (Vettor et al., 1997). Leptin levels may reflect fat mass, which is decreased by TES treatment (Isidori et al., 2000). Moreover, leptin exerts receptor-mediated inhibitory action on hCG-stimulated TES secretion *in vitro* from Leydig cells in rat (Caprio et al., 1999). There may be a threshold level for serum TES that is necessary for the suppression of leptin. To indicate this negative relationship, some authors found that administration of TES reduced leptin levels in hypo-gonadal (Jockenhövel et al., 1997; Sih et al., 1997). During puberty, high TES levels were found to parallel a reduction in level of leptin (Garcia-Mayor et al., 1997; Mantzoros et al., 1997). Suppression of TES in boys by GnRH agonist treatment increased leptin levels (Palmert et al., 1998). Since TES is involved in the regulation of fat mass, it may be speculated an indirect relation between TES and leptin (Söderberg et al., 2001). The recent results of Lima et al. (2020) indicated

that increasing level of serum leptin is independently related to reducing TES level, even when controlling for other associated factors. In this context, Isidori et al. (1999) studied the relationship between leptin and reproductive hormone levels. There was an adverse correlation between androgen and leptin levels after hCG stimulation (Garcia et al., 2002). Leydig cells have leptin receptors (Caprio et al., 1999) and high serum leptin levels could actively impair (Alberti et al., 2005; Mulhall et al., 2018), lyase steps of the testicular steroidogenic pathway, causing reduced production of intra-testicular TES (Caprio et al., 1999).

Throughout aging, the pituitary gland has an ability to adapt its cellular contents to fulfill altering the body endocrine demands. The anterior pituitary contains lactotropes cells which produce PRL, playing an essential role in pregnancy and lactation (Vankelecom, 2016). During postnatal life, the pituitary gland has the flexibility to modulate the production of its hormones by re-modulation its activity and cellular contents in these conditions (Styne, 2003). The pituitary gland in rodent almost doubles in size several weeks after birth in term of increasing cell proliferation and expansion of cell size because of endocrine differentiation with accumulation of hormone-containing secretory granules (Zhu et al., 2015). The effect of age and weight on PRL levels of males in human was reported by Isidori et al. (2000), who found serum PRL was 3.5±0.8 and 4.0±0.7 ng/ml at <30 vs. >30 BMI, respectively. In females, the rise in PRL is substantially due to an increase of lactotropes number in the anterior pituitary (Laporte et al., 2021). In a study of Roelfsema et al. (2012), age was weakly negatively correlated with PRL pulse mass. PRL secretion during the daytime was age-invariant (Iranmanesh et al., 1999) suggesting that the decrease in nocturnal PRL secretion is not the result of decreased lactotrope cell mass. Generally, rate of increase in TES, leptin and PRL levels between 1 and 24 month of age was about 4800, 254, and 870%, respectively. This was reflected in higher and significant ($P<0.01$) positive correlation coefficients between TES-leptin, TES-PRL, and leptin-PRL correlations, being 0.797, 0.859, and 0.973, respectively.

CONCLUSION

Based on these findings, the present results may suggest an increase in levels of all hormones (TES, leptin, and PRL) depending on age progress or change in body weight of Egyptian buffalo males. Such results also indicated more acceleration in TES level than in PRL and leptin levels by advancing age and/or weight of buffalo-male bulls.

ACKNOWLEDGMENTS

The authors thank Animal Production Experimental Stations of Mahalet Mousa, Animal Production Research Institute, Egypt for allowing animals that used in this study.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

NOVELTY STATEMENT

According to the results of blood testosterone, leptin, and prolactin concentrations at different age interval, it may be illustrate the relationships between these hormones in Egyptian buffalo males with advancing age from one to 24 months.

AUTHORS CONTRIBUTIONS

All authors were contributed to design the experimental work. Wafa, W.M. and Sakr, A.M. were conducted the experimental procedures and collected data. Hegazy, M.M. and El-Nagar, H.A. were performed the sample preparations for laboratory analysis. Wafa, W.M. conducted the statistical analyses. Wafa, W.M. and El-Nagar, H.A. were drafted the manuscript reviewed the draft paper and delivered recommendations.

REFERENCES

Ahrén B, Pacini G (1998). Age-related reduction in glucose elimination is accompanied by reduced glucose effectiveness and increased hepatic insulin extraction in man. J. Clin. Endocrinol. Metab., 83(9): 3350-3356. https://doi.org/10.1210/jcem.83.9.5107.

Alberti KG, Zimmet P, Shaw J; IDF Epidemiology Task Force Consensus Group (2005). The metabolic syndrome-a new worldwide definition. Lancet., 366(9491): 1059-1062. https://doi.org/10.1016/S0140-6736(05)67402-8.

Al-Qarawi AA, Omar HM, Abdel-Rahman HA, El-Mougy SA, El-Belely MS. (2004). Trypanosomiasis-induced infertility in dromedary (Camelus dromedarius) bulls: changes in plasma steroids concentration and semen characteristics. Anim. Reprod. Sci., 84: 73-82. https://doi.org/10.1016/j.anireprosci.2003.10.013.

Aquila S, Gentile M, Middea M, Catalano S, Morelli C, Pezzi V (2005). Leptin secretion by human ejaculated spermatozoa. J. Clin. Endocrinol. Metab., 90 (8): 4753-4761. https://doi.org/10.1210/jc.2004-2233.

Baumgartner RN, Waters DL, Morley JE, Patrick P, Montoya GD, Garry PJ (1999). Age-related changes in sex hormones affect the sex difference in serum leptin independently of changes in body fat. Metabolism.,48(3): 378-384. https://doi.org/10.1016/s0026-0495(99)90089-6.

Bezerra FQG, Aguiar Filho CR, Freitas Neto LM, Santos Junior ER, Chaves RM, Azevedo EMP, Santos MHB, Lima PF, Oliveira MAL (2009). Body weight, scrotal circumference and testosterone concentration in young Boer goat males born during the dry or rainy seasons. South African Journal of Animal Science, 39 (Issue 4):301-306. https://doi.

org/10.4314/sajas.v39i4.51123.

Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Müller JR, Skakkebaek NE, Heiman ML, Birkett M, Attanasio A, Kiess W, Rascher W (1997). Plasma leptin levels in healthy children and adolescent: dependence in body mass index, body fat mass, gender, pubertal stage and testosterone. J. Clin. Endocrinol. Metabol. 82 (9): 2904-2910. https://doi.org/10.1210/JCEM.82.9.4251.

Bray GA, York DA (1997). Clinical review 90: Leptin and clinical medicine: a new piece in the puzzle of obesity. J. Clin. Endocrinol. Metabol. 82 (9): 2771-2776 . https://doi.org/10.1210/JCEM.82.9.4224.

Caprio M, Isidori AM, Carta AR, Moretti C, Dufau ML, Fabbri A (1999). Expression of functional leptin receptors in rodent Leydig cells. Endocrinology, 140(11): 4939-4947. https://doi.org/10.1210/endo.140.11.7088.

Chou SH, Mantzoros C (2014). 20 years of leptin: Role of leptin in human reproductive disorders. J. Endocrinol. 223 (1): T49-T62. https://doi.org/10.1530/JOE-14-0245.

Delgadillo JA, Chemineau P. (1992). Abolition of the seasonal release of luteinizing hormone and testosterone in Alpine male goats (Capra hircus) by short photoperiodic cycles. J. Reprod. Fertil., 94(1): 45-55. https://doi.org/10.1530/jrf.0.0940045.

Dietrich MO, Spuch C, Antequera D, Rodal I, de Yébenes JG, Molina JA, Bermejo F, Carro E (2008). Megalin mediates the transport of leptin across the blood-CSF barrier. Neurobiol. Aging., 29(6): 902-912. https://doi.org/10.1016/j.neurobiolaging.2007.01.008.

Duncan DB (1955). "Multiple range and multiple F tests". Biometrics., 11: 1-42. https://doi. org/10.2307/3001478.

FAO (2017). BEFS Assessment for Egypt: Sustainable bioenergy options from crop and livestock residues. Food and Agriculture Organization. http://www.fao.org/3/a-i6684e.pdf.

Fernández-Galaz C, Fernández-Agulló T, Campoy F, Arribas C, Gallardo N, Andrés A, Ros M, Carrascosa JM (2001). Decreased leptin uptake in hypothalamic nuclei with ageing in Wistar rats. J. Endocrinol., 171(1): 23-32. https://doi.org/10.1677/joe.0.1710023.

Fischer H, Bodhipaksha P (1992). Reproduction in swamp buffaloes. In: Buffalo Production. Tulloh NM, Holmes JHG, (eds.). 1st edition, Elsiever Science Publisher, Amesterdam, Netherland. ISBN: 0444880763 9780444880765.

Garcia MR, Amstalden M, Williams SW, Stanko RL, Morrison CD, Keisler DH, Nizielski SE, Williams GL (2002). Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. J. Anim. Sci., 80(8): 2158-2167. https://doi.org/10.2527/2002.8082158x.

Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF (1997). Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J. Clin. Endocrinol. Metab., 82(9): 2849-2855. https://doi.org/10.1210/jcem.82.9.4235.

Garg SL, Sharma Shrdha, Rose MK, Agarwal VK (2002). Age associated variations in peripheral concentration of certain hormones of female buffalo calves from birth to puberty. Indian J. Anim. Sci., 72: 579-581.

Gholami H, Towhidi A, Zare Shahneh A, Dirandeh E (2010). The relationship of plasma leptin concentration and puberty in Holstein bull calves (Bos taurus). J Anim Physiol Anim Nutr (Berl), 94(6): 797-802. https://doi.org/10.1111/j.1439-0396.2009.00970.x.

Glander HJ, Lammert A, Paasch U, Glasow A, Kratzsch J (2002). Leptin exists in tubuli seminiferi and in seminal plasma. Andrologia., 34(4): 227-33. https://doi.org/10.1046/j.1439-0272.2002.00501.x.

Goeritz F, Quest M, Wagener A, Fassbender M, Broich A, Hildebrandt TB, Hofmann RR, Blottner S (2003). Seasonal timing of sperm production in roe deer: interrelationship among changes in ejaculate parameters, morphology and function of testis and accessory glands. Theriogenology, 59 (7): 1487-502. https://doi.org/10.1016/S0093-691X(02)01201-3.

Gonzales GF, Velasquez G, Garcia-Hjarles M (1989). Hypoprolactinemia as related to seminal quality and serum testosteron. Arch. Androl., 23(3): 259-265. https://doi.org/10.3109/01485018908986849.

Gordon I (1996). Controlled Reproduction in Cattle and Buffaloes. 1st edition, CAB International, Willingford, UK.PP. 432-466. ISBN: 0851991149 9780851991146.

Habeeb AAM, Gad AE, Atta MAA (2016). Changes in Body Weight Gain and Blood Hormonal Levels in Relation to Change in Age of Egyptian Male Buffaloes Calves from Birthing to Puberty. Advances in Applied Physiology, 1 (4): 43-48. https:// doi.org/10.11648/j.aap.20160104.11.

Hadley ME (2000). Endocrinology, Prentice Hall, Inc., Upper Saddle River, New Jersey, pp 97-99, 517-518, 525, www.biblio.com/97801308.

Hafez ESE, Hafez B (2000). Reproduction in Farm Animals: Reproductive Cycle in Farm Animals, 7th edn. Lippincott Williams and Wilkins, Philadelphia, USA. ISBN: 978-1-118-71028-9.

Hernández Uribe L, Hernández Marín I, Cervera-Aguilar R, Ayala AR (2001). Frecuencia y etiología de azoospermia en el estudio de parejas infértiles [Frequency and etiology of azoospermia in the study of infertile couples]. Ginecol. Obstet. Mex., 69: 322-326. Spanish. PMID: 11599318.

IBM Corp. Released (2017). IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. (https://www.ibm.com/support/pages/how-cite-ibm-spss-statistics-or-earlierversions-spss).

Ingole SD, Deshmukh BT, Nagvekar AS, Bharucha SV (2012). Serum Profile of Thyroid Hormones from Birth to Puberty in Buffalo Calves and Heifers. Journal of Buffalo Science, 1: 39-49. https:// doi.org/10.6000/1927-520X.2012.01.01.08.

Iranmanesh A, Mulligan T, Veldhuis JD (1999). Mechanisms subserving the physiological nocturnal relative hypoprolactinemia of healthy older men: dual decline in prolactin secretory burst mass and basal release with preservation of pulse duration, frequency, and interpulse interval--a General Clinical Research Center study. J. Clin. Endocrinol. Metab., 84(3): 1083-1090. https://doi.org/10.1210/jcem.84.3.5514.

Isidori AM, Caprio M, Strollo F, Moretti C, Frajese G, Isidori A, Fabbri A (1999). Leptin and androgens in male obesity: evidence for leptin contribution to reduced androgen levels. J. Clin. Endocrinol. Metab., 84(10): 3673-3680. https://doi.org/10.1210/jcem.84.10.6082.

Isidori AM, Strollo F, Morè M, Caprio M, Aversa A, Moretti C, Frajese G, Riondino G, Fabbri A (2000). Leptin and aging: correlation with endocrine changes in male and female healthy adult populations of different body weights. J. Clin. Endocrinol. Metab., 85(5):1954-62. https://doi.

org/10.1210/jcem.85.5.6572.

Jockenhövel F, Blum WF, Vogel E, Englaro P, Müller-Wieland D, Reinwein D, Rascher W, Krone W (1997). Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. J. Clin. Endocrinol. Metab., 82(8): 2510-2513. https://doi.org/10.1210/jcem.82.8.4174.

Kawamura K, Sato N, Fukuda J, Kodama H, Kumagai J, Tanikawa H, Nakamura A, Tanaka T (2002). Leptin promotes the development of mouse preimplantation embryos in vitro. Endocrinology, 143 5, 1922-1931. https://doi.org/10.1210/ENDO.143.5.8818.

Laporte E, Vennekens A, Vankelecom H (2021). Pituitary Remodeling Throughout Life: Are Resident Stem Cells Involved? Front Endocrinol (Lausanne)., 11: 604519. https://doi.org/10.3389/fendo.2020.604519.

Lima TFN, Nackeeran S, Rakitina E, Lima GFN, Arora H, Kargi AY, Ramasamy R (2020). Association of Leptin with Total and Free Testosterone: Results from the National Health and Nutrition Examination Surveys. Androg. Clin. Res. Ther., 1(1): 94-100. https://doi.org/10.1089/andro.2020.0007.

Luboshitzky R, Kaplan-Zverling M, Shen-Orr Z, Nave R, Herer P (2002). Seminal plasma androgen/estrogen balance in infertile men. Int. J. Androl. 25(6):345-51. https://doi.org/10.1046/j.1365-2605.2002.00376.x.

Malfatti A, Barbato O, Todini L, Terzano GM, Debenedetti A, Borghese A (2006). Blood testosterone levels in Italian Mediterranean buffalo bulls managed in two different breeding conditions. Theriogenology., 65: 1137-1144. https://doi.org/10.1016/j.theriogenology.2005.07.012.

Mantzoros CS, Flier JS, Rogol AD (1997). A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J. Clin. Endocrinol. Metab., 82(4): 1066-1070. https://doi.org/10.1210/jcem.82.4.3878.

Matsuzaki S, Uenoyama Y, Okuda K, Watanabe G, Kitamura N, Taya K, Yamada (2000). Age-Related Changes in the Serum Levels of Inhibin, FSH, LH and Testosterone in Holstein Bulls. J. Reprod. Develop., 46(4): 245-248. https://doi.org/10.1262/jrd.46.245.

Mulhall JP, Trost L, Brannigan RE, Kurtz EG, Redmon B, Chiles KA, Lightner DJ, Miner M, Murad H, Nelson CJ, Platz EA, Ramanathan LV, Lewis RW (2018). Evaluation and Management of Testosterone Deficiency: AUA Guideline. J. Urol. 200, 423-432. https://doi.org/10.1016/j.juro.2018.03.115.

Nett TM (1993). Reproductive peptide and protein hormones. Page 110 in: Equine Reproduction. A. McKinnon and J. Voss, ed. Williams & Wilkins, Media, PA.

Official Journal of the European Union. (2010). Directive 2010/63/ EU of 22 September 2010 on the protection of animals used for scientific purposes. L276/33-792010. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

Ostlund RE Jr, Yang JW, Klein S, Gingerich R (1996). Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J. Clin. Endocrinol. Metab., 81(11): 3909-3913. https://doi.org/10.1210/jcem.81.11.8923837.

Palmert MR, Radovick S, Boepple PA (1998). The impact of reversible gonadal sex steroid suppression on serum leptin concentrations in children with central precocious puberty. J. Clin. Endocrinol. Metab., 83(4):1091-1096. https://doi.org/10.1210/jcem.83.4.4700.

Pasqualotto FF, Lucon AM, de Góes PM, Sobreiro BP, Hallak J, Pasqualotto EB, Arap S (2005) Semen profile, testicular volume, and hormonal levels in infertile patients with varicoceles compared with fertile men with and without varicoceles. Fertil. Steril., 83(1): 74-77. https://doi.org/10.1016/j.fertnstert.2004.06.047.

Pétervári E, Rostás I, Soós S, Tenk J, Mikó A, Füredi N, Székely M, Balaskó M (2014). Age versus nutritional state in the development of central leptin resistance. Peptides., 56: 59-67. https://doi.org/10.1016/j.peptides.2014.03.011.

Roelfsema F, Pijl H, Keenan DM, Veldhuis JD (2012). Prolactin secretion in healthy adults is determined by gender, age and body mass index. PLoS One., 7(2): e31305. https://doi.org/10.1371/journal.pone.0031305.

Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, Leibel RL (1996). Effects of gender, body composition, and menopause on plasma concentrations of leptin. J. Clin. Endocrinol. Metab., 81(9): 3424-3427. https://doi.org/10.1210/jcem.81.9.8784109.

Sánchez-Garrido MA, Tena-Sempere M (2013). Metabolic control of puberty: Roles of leptin and kisspeptins. Hormones Behav., 64, 187-194. https://doi.org/10.1016/j.yhbeh.2013.01.014

Scarpace PJ, Matheny M, Tumer N (2001). Hypothalamic leptin resistance is associated with impaired leptin signal transduction in aged obese rats. Neuroscience, 104(4): 1111-1117. https://doi.org/10.1016/S0306-4522(01)00142-7.

Schultheiss D, Hiltl DM, Meschi MR, Machtens SA, Truss MC, Stief CG, Jonas U (2000). Pilot study of the transdermal application of testosterone gel to the penile skin for the treatment of hypogonadotropic men with erectile dysfunction. World J. Urol. 18, 431–435. https://doi.org/10.1007/PL00007086.

Segal S, Yaffe H, Laufer N, Ben-David M (1979). Male hyperprolactinemia: Effects on fertility. Fertil. Steril., 32(5): 556-561. https://doi.org/10.1016/s0015-0282(16)44359-1.

Sih R, Morley JE, Kaiser FE, Perry HM 3rd, Patrick P, Ross C (1997). Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J. Clin. Endocrinol. Metab., 82(6): 1661-1667. https://doi.org/10.1210/jcem.82.6.3988.

Singh Shatab M, Kumar A, Honparkhe M, Singhal S, Kaur S, Singh Brar P (2016). Endocrine Status of Serum Testosterone, Estrogen and Thyroid Hormones in High Fertility Breeding Buffalo Bulls and their Male Calves. Journal of Animal Research, 6(6): 1073-1075. https://doi.org/10.5958/2277-940X.2016.00158.3.

Söderberg S, Olsson T, Eliasson M, Johnson O, Brismar K, Carlström K, Ahrén B (2001). A strong association between biologically active testosterone and leptin in non-obese men and women is lost with increasing (central) adiposity. Int. J. Obes. Relat. Metab. Disord., 25(1): 98-105. https://doi.org/10.1038/sj.ijo.0801467.

Steinman N, Gamzu R, Yogev L, Botchan A, Schreiber L, Yavetz H (2001). Serum leptin concentrations are higher in azoospermic than in normozoospermic men. Fertil. Steril., 75(4): 821-822. https://doi.org/10.1016/s0015-0282(01)01707-1.

Styne DM (2003). The regulation of pubertal growth. In: Hormone Research. Basel, Switzerland: Karger Publishers., p. 22-26. https://doi.org/10.1159/000071222.

Swelum AA, Saadeldin IM, Zaher HA, Alsharifi SAM, Alowaimer AN (2017). Effect of sexual excitation on testosterone and

nitric oxide levels of water buffalo bulls (Bubalus bubalis) with different categories of sexual behavior and their correlation with each other. Anim. Reprod. Sci., 181: 151-158. https://doi.org/10.1016/j.anireprosci.2017.04.003. Epub 2017 May 4.

Tena-Sempere M, Barreiro ML, Lage M, Dieguez C, Casanueva FF. (2007). Role of leptin and ghrelin in the regulation of gonadal function. Expert. Rev. Endocrinol. Metab., 2 (2): 239-249. https://doi.org/10.1586/17446651.2.2.239.

Vankelecom H (2016). "Pituitary stem cells: Quest for hidden functions. In: Research and Perspectives in Endocrine Interactions. Cham, Switzerland: Springer Verlag., p. 81-101. https://doi.org/10.1007/978-3-319-41603-8_7

Vettor R, De Pergola G, Pagano C, Englaro P, Laudadio E, Giorgino F, Blum WF, Giorgino R, Federspil G (1997). Gender differences in serum leptin in obese people: relationships with testosterone, body fat distribution and insulin sensitivity. Eur. J. Clin. Invest., 27(12): 1016-1024. https://doi.org/10.1046/j.1365-2362.1997.2270773.x

Vignozzi L, Corona G, Petrone L, Filippi S, Morelli A, Forti G, Maggi M (2005). Testosterone and sexual activity. J. Endocrinolog. Investig., 28 (3) Suppl.: 39-44.

Wabitsch M, Blum WF, Muche R, Braun M, Hube F, Rascher W, Heinze E, Teller W, Hauner H (1997). Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J. Clin. Invest., 100(4): 808-813. https://doi.org/10.1172/JCI119595.

Wauters M, Considine RV, Van Gaal LF (2000). Human leptin: from an adipocyte hormone to an endocrine mediator. Eur. J. Endocrinol., 143(3): 293-311. https://doi.org/10.1530/eje.0.1430293.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994). Positional cloning of the mouse obese gene and its human homologue. Nature, (372): 425-432. https://doi.org/10.1038/372425A0.

Zhu X, Tollkuhn J, Taylor H, Rosenfeld MG (2015). Notch-dependent pituitary SOX2+stem cells exhibit a timed functional extinction in regulation of the postnatal gland. Stem Cell Rep. 5:1196-1209. https://doi.org/10.1016/j.stemcr.2015.11.001.