



## Effects of Age and Season on Serum Testosterone Level in Male Buffaloes

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### ABSTRACT

The aim of the present study was to detect the changes occurring in serum testosterone profile in male buffaloes. Thirty blood samples from apparently healthy slaughtered male buffaloes were taken and divided into three age groups, 1.5–1.8, 2–2.5 and 3–4 years. Scrotal circumference and testicular measurements were conducted and the seminal glands were obtained immediately after slaughter. The fructose content was determined in tissue of seminal gland using spectrophotometer. Our investigations were extended to determine the effect of the seasons on serum testosterone levels (Indoor study). There were significant differences between scrotal circumference, testicular and seminal glands measurements with the age of the animals. There were no significant differences neither between average fructose content of seminal glands (mg/ gland) nor fructose concentration per 1 g. tissue with age, were detected. In addition, it is noticed that the serum testosterone level was higher in the first group (1.5–1.8 years), then a decline in testosterone levels was recorded from 2.0–2.5 to 3–4 years of age with no significant difference between the different groups. A higher mean testosterone concentration (1.72 ng/ ml) was recorded in autumn, while the lowest average concentration (0.77 ng / ml) was recorded in winter. However, there was no significant difference in testosterone levels between different seasons of the year. Hence, we could suppose that the Egyptian water buffalo bull has no typical breeding season.

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### Introduction

The world population of domestic water buffalo was estimated to be about 206 million, in which more than 201 million (97%) of this number is located in Asia, and about 3.5 million (1.7%) is located in Africa, almost entirely in Egypt (FAO, 2018). The water buffaloes are considered the main milk, meat and hide producing animals. In Egypt, buffaloes represent a significant part of the domestic livestock and constitute a major part of the dairy industry.

Testosterone, which is the primary male sex hormone is produced by Leydig cells. It is essential for the onset and maintenance of spermatogenesis, development and maintenance of mature sexual behavior. Also, it plays an important role in growth, development, and secretory activity of the accessory sex glands and stimulates secondary sexual characteristics that comprising the male phenotype (Hafez *et al.*, 2000).

The seminal gland is one of the most important male accessory genital glands in the body. Secretion from seminal glands forms the majority of the seminal plasma (Juyena and

Stelletta, 2012). Its fructose content is related to the levels of circulating testosterone (Mann and Lutwak-Mann, 1981; Shinde, 2013).

The different dimensions of the testis including its calculated volume play a major role in the rate of sperm production in buffalo bull (Osman, 1972). Moreover, estimation of scrotal circumference and testicular parameters is an essential part of breeding soundness evaluation of breeding bulls as reported by Pant *et al.* (2003) and Elmaz *et al.* (2008). Furthermore, testicular size and scrotal measurements can be used as indicators for reproductive capacity and spermatogenic capabilities in the post-pubertal breeding bulls (Ahmad *et al.*, 1989; Perumal, 2014). Serum testosterone concentration in buffalo bulls varies with the season and the time of the day (Barnabé *et al.*, 1995). The aim of this study was to throw some lights on the event of changes occurring in serum testosterone level in relation to the age, season, testicular size and the seminal gland biometries and its fructose contents in male buffalo.

### Materials and methods

#### Ethical statement

Animal experiments described in this study were con-

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ducted in accordance with the ethical animal guidelines and regulations set by the Animal Care Committee of Assiut University, Egypt. The study is a part of Master Thesis at Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, Egypt.

#### *Experimental design*

##### *Effect of age on testis measurements, seminal glands biometry and testosterone level (slaughterhouse materials)*

Thirty (30) blood samples from slaughtered male buffaloes were taken and divided into three sub-groups according to the age: 1.5 – 1.8, 2.0 – 2.5 and 3.0 – 4.0 years. The age of the studied buffalo bulls was determined as accurately as possible from their teeth according to their dental formula as described by Payne and Wilson (1999) just before slaughtering in a local abattoir in Assiut Governorate, Egypt, at the early morning. Seminal glands were also taken for further investigation. All buffalo bulls were apparently healthy, free from infectious diseases and their meat were fit for human consumption. The live body weights of the bulls were recorded.

##### *Scrotal circumference*

In order to determine the scrotal circumference of each buffalo bull before the slaughtering, the testes were brought into the distal part of the scrotum until the ventral scrotal skin folds. Testes were then held firmly in place by grasping the scrotum with left hand above the head of the epididymis. The right hand was then used to guide the loop of a flexible measuring tape around the greatest diameter of the scrotum. Scrotal circumference then measured with sufficient manual pressure on the measuring tape to cause slight skin indentation (Coulter, 1991; Chenoweth et al., 2010).

##### *Collection and handling of materials*

Blood samples were taken from the Jugular vein of the bled animals in a clean plain blood collecting tubes and covered firmly. The tubes were put in closed container and surrounded by ice. The blood was rapidly transported to the laboratory and centrifuged at 3000 rpm for 10 minutes. Serum was aspirated by clean sterile micropipette and stored frozen at -20°C till hormone assay.

##### *Testicular Measurements*

Testicular length, width and depth were measured by caliper after Osman (1970). Testicular length was measured by placing the fixed arm of the caliper at the upper pole and the sliding arm at the lower pole of the testes. Care was taken to exclude the epididymis. Depth (antro-posterior) was measured by placing the fixed arm of the caliper at the anterior aspect of each testis and the sliding arm at the posterior aspect, at the point of maximum depth. Width or breadth (from lateral to lateral) was measured by placing one arm of the caliper at the medial aspect of each testis and the other at the lateral free aspect, at the point of maximum width. Testicular volume was calculated using the following formula: Volume (cm<sup>3</sup>)= length (L)×width (W)×Depth (D)×0.52.

##### *Examination of the Seminal gland*

##### *Weight and biometry*

The pelvic genitalia including the seminal glands were obtained immediately after slaughter, kept in a closed container

and surrounded by ice and transported rapidly to the laboratory. Each seminal gland (right and left) was dissected carefully and removed from its surrounding peritonium, fascia and separated at the point where the collecting ducts passes under the body of prostate gland, then each gland weighed in grams using a digital balance (Precision balance KB-N).

Seminal gland biometry measured using centimeter ruler and caliper. Length (distance from the urethral attachment to the free pole), breadth (distance from the medial to the lateral border), thickness (distance from dorsal to ventral surface). After that, tissues of the seminal glands were kept at -20°C until fructose determination.

##### *Determination of fructose in seminal gland tissue*

Fructose was determined in tissue extracts according to the method of Lindner and Mann (1960). In brief, 0.5 - 1g of the glandular portion of the seminal gland was grounded in a mortar with 8 ml ethanol (80%) and some sand. Then the pulp was centrifuged. The residue was dispersed in 3 ml, distilled water, then grounded again with 8 ml of absolute ethanol and recentrifuged. The combined supernatant fluids were concentrated in an evaporating dish on a water bath to a volume of 4 ml. The contents of the dish were transferred after washing several times with water into a graduating centrifuge tube and the followings were added to it: 1 ml barium hydroxide 0.3 N (Oxford lab fine chem LLP) and 1 ml 5% zinc sulphate (ADWIC). The volume was completed to 10 ml with distilled water and centrifuged again. From the clear protein free supernatant solution, 1 ml was taken in a test tube and the followings were added to it: 1 ml distilled water, 2 ml alcoholic resoreinol 0.1 % (Biotec) and 6 ml 30 % (w/v) hydrochloric acid. The tubes were heated for 10 minutes in a water bath at 90-92°C and after cooling the fructose present was determined in spectrophotometer (Optizen 3220 UV Spectrophotometer, Mecasys, Korea) at 540 nm. The results were calculated from a calibration curve prepared with standard solutions of pure fructose.

##### *Determination of serum testosterone level*

Serum testosterone level was determined by using testosterone Enzyme immunoassay (EIA) test Kit (Catalogue Number : BC-1115, BioCheck, Inc S. San Francisco, CA 94080, USA ). The minimum detectable concentration of the BioCheck Testosterone ELISA assay, as measured by 2 SD from the mean of a zero standard, is estimated to be 0.05 ng/ml.

The testosterone concentration was calculated according to the commercial Kit. The mean absorbance value (A450) for each set of reference standards, controls and samples were determined. A standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis. Applying the mean absorbance values for each specimen to determine the corresponding concentration of Testosterone in ng/ml from the standard curve.

##### *Seasonal effect on serum testosterone level (Indoor study)*

In order to investigate the effect of seasons on serum testosterone levels, four adult buffalo bulls raised in the Veterinary Teaching Hospital, Assiut University, Egypt, were used. Blood samples were taken monthly (from June 2018 to May 2019). Sera were obtained by centrifugation and kept at -20°C for testosterone determination. Biometry of the testes and the seminal glands have been measured clinically at the day of blood sampling (Osman, 1970; 1971).

Statistical analysis

Statistical analysis of the obtained data was conducted with the Statistical Package for Social Science computer program (IBM SPSS version 21, Armonk, NY). Mean values ( $\pm$ SE) were calculated from the raw data, then analyzed by one way ANOVA between the different age groups, and the difference between mean values was compared through Duncan's multiple range test and LSD ( $P < 0.05$ ) to determine significant differences between the different age groups. Differences with values of  $P < 0.05$  were considered to be statistically significant. Differences at  $P < 0.05$  and  $P < 0.01$  were considered to be statistically significant.

Results

Effect of age on testis measurements, seminal glands biometry and testosterone level (slaughterhouse materials)

Scrotal circumference and testicular biometry

The scrotal circumference and testicular biometry were increased with the age. There were significant differences ( $p \leq 0.05$ ) between scrotal circumference and testicular measurements with the age. On the other hand, there was a significant difference in body weight between 1.5–1.8 and 2.0–2.5 years (Table 1).

Seminal glands

Biometry

Among seminal glands biometry, Table (2) clarified the different measurements (length, breadth, thickness and weight) of the right and left seminal glands as related to age groups. It is clear that the total seminal glands weight increased with the advancement of age. The breadth, thickness and weight of both right and left seminal glands increased also with age.

However, the length of the right seminal gland decreased with the progression of age. The length of the left seminal gland decreased from 1.5-1.8 to 2.0–2.5 years and re-increased again at 3 -4 years of age but not at a significant level. Moreover, there was a slight difference between the left and right seminal gland in the term of biometry.

Fructose concentration in seminal gland tissue

The results recorded for the fructose concentrations were expressed in mg per 1 gram of tissue as well as per total seminal glands were summarized in Table (3). The fructose content of seminal glands (mg / gland) increased with the growths of seminal gland (weight) from 1.5 to 4 years of age.

Serum testosterone concentrations

Average serum testosterone concentration in different age groups of slaughtered buffalo bulls was mentioned in Table (4). It was noticed that the serum testosterone level was higher in the first group (1.5–1.8 y), then declined in 2.0 –2.5 and 3.0 – 4.0 years of age. However, there was no significant difference between the different groups.

Seasonal effect on serum testosterone level

The results reported for serum testosterone variations during different seasons of the year are presented in Table (5). It was clear that the higher mean testosterone concentration (1.72 ng/ml) was recorded in autumn, followed by summer and spring, while the lowest average concentration (0.77 ng/ml) was recorded in winter. Individual variations in testosterone levels among different seasons of the year were present. A maximum value of 4.45 ng/ml was reported in the summer, whereas the minimum value of 0.13 ng/ml was recorded in winter. Statistically, there is no significant difference in testosterone levels between different seasons of the year.

Table 1. Live body weight, scrotal circumference and testicular biometry of slaughtered male buffaloes at different age groups

Age groups (Years)	n.	Live Body Weight (Kg)	Scrotal circumference (cm)	Testicular biometry			
				Length (cm)	Depth (cm)	Breadth (cm)	Volume (cm <sup>3</sup> )
1.5 -1.8	10	347 $\pm$ 19.43 <sup>b</sup>	23.55 $\pm$ 0.24 <sup>c</sup>	6.52 $\pm$ 0.094 <sup>c</sup>	4.22 $\pm$ 0.087 <sup>c</sup>	3.84 $\pm$ 0.076 <sup>c</sup>	55.22 $\pm$ 2.92 <sup>c</sup>
2.0 - 2.5	16	430.62 $\pm$ 13.27 <sup>a</sup>	25.89 $\pm$ 0.25 <sup>b</sup>	7.237 $\pm$ 0.085 <sup>b</sup>	4.57 $\pm$ 0.098 <sup>b</sup>	4.2 $\pm$ 0.088 <sup>b</sup>	73.21 $\pm$ 3.75 <sup>b</sup>
3.0 – 4.0	4	435.5 $\pm$ 15.54 <sup>a</sup>	28.87 $\pm$ 0.59 <sup>a</sup>	8.5 $\pm$ 0.51 <sup>a</sup>	5.15 $\pm$ 0.15 <sup>a</sup>	4.67 $\pm$ 0.13 <sup>a</sup>	107.57 $\pm$ 12.42 <sup>a</sup>

Data were expressed as Mean $\pm$ St.Er

<sup>a,b,c</sup>Different superscript letters in the same column are considered significantly different at  $P < 0.05$ .

Table 2. Relation between seminal gland measurements as measured anatomically and age from slaughtered male buffaloes.

Age groups (Years)	n.	Seminal gland measurements								Total weight (g)
		Left				Right				
		Length (cm)	Breadth (cm)	Thickness. (cm)	Weight (g)	Length (cm)	Breadth (cm)	Thickness. (cm)	Weight (g)	
1.5 -1.8	10	5.76 $\pm$ 0.44	2.91 $\pm$ 0.21	0.97 $\pm$ 0.089	7.96 $\pm$ 0.88	5.59 $\pm$ 0.34	2.99 $\pm$ 0.14	0.91 $\pm$ 0.082	7.36 $\pm$ 0.69	15.25 $\pm$ 1.58
2.0 - 2.5	16	5.68 $\pm$ 0.48	2.96 $\pm$ 0.16	1.012 $\pm$ 0.107	9.048 $\pm$ 1.86	5.475 $\pm$ 0.49	3.025 $\pm$ 0.17	0.95 $\pm$ 0.109	8.85 $\pm$ 1.99	17.903 $\pm$ 3.84
3.0 – 4.0	4	5.7 $\pm$ 0.63	3.13 $\pm$ 0.51	1.05 $\pm$ 0.25	9.23 $\pm$ 1.57	5.325 $\pm$ 0.63	3.325 $\pm$ 0.39	1.125 $\pm$ 0.94	9.802 $\pm$ 2.26	19.03 $\pm$ 3.84

Data were expressed as Mean $\pm$ St.Er

Table 3. Relation between fructose concentrations in seminal gland tissue and age from slaughtered male buffaloes.

Age groups (Years)	Total seminal glands weight (g)	Fructose (mg/g tissue)	Total fructose (mg/gland)
1.5 -1.8	15.254 $\pm$ 1.58	2.38 $\pm$ 0.15	37.28 $\pm$ 5.13
2.0 - 2.5	17.903 $\pm$ 3.84	2.63 $\pm$ 0.22	46.61 $\pm$ 11.32
3.0 – 4.0	19.03 $\pm$ 3.84	3.09 $\pm$ 0.39	61.49 $\pm$ 18.51

Data were expressed as Mean $\pm$ St.Er

Table 4. Average testosterone concentration (ng / ml) in different age groups from slaughtered male buffaloes

Age groups (Years)	Testosterone concentration (ng/ml)	
	Mean $\pm$ S.E.	
1.5 -1.8	0.991 $\pm$ 0.28	
2.0 - 2.5	0.62 $\pm$ 0.37	
3.0 - 4.0	0.26 $\pm$ 0.001	

Table 5. Seasonal variations in serum testosterone concentrations (ng/ml) in buffalo bull

Season	n.	Testosterone concentrations (ng/ml)		
		Mean $\pm$ S.E.	minimum	maximum
Winter	9	0.776 $\pm$ 0.34	0.13	3.28
Spring	11	1.012 $\pm$ 0.28	0.14	2.65
Summer	12	1.425 $\pm$ 0.35	0.18	4.45
Autumn	7	1.720 $\pm$ 0.61	0.14	4.25
Overall average	39	1.212 $\pm$ 0.19	0.13	4.45

## Discussion

In Egypt, water buffalo represents a significant part of animal production. With regard to scrotal circumference of slaughtered male buffalo, the obtained results were comparable to those reported by *Ahmad et al. (1984)*. The average scrotal circumference at 18–20 months and 3–4 years in the present work were similar to those obtained by *Pant et al. (2003)* at the same ages in Murrah buffalo. On the other hand, a much lower scrotal circumference values in swamp buffalo were reported by *Bongso et al. (1984)* at the same ages, with maximum scrotal circumference (26 cm) at 48.5 months of age compared with 30.5 cm at the same age in the present study. The average scrotal circumference recorded in the present study was higher than those reported by *Henry et al. (2018)* and *Mahmood et al. (2018)* in buffalo bulls.

*Osman (1972)* reported higher values for testicular volume as estimated by water displacement in buffalo bulls at 1.5 years and 2.5 years, also *Ahmad et al. (1989)* reported higher values at 22–24 months. Moreover, In Murrah buffalo bull, *Pant et al. (2003)* reported higher values at 25–36 and 37–48 months. However, *da Luz et al. (2013)* reported lower values for, testicular length, width, and breadth in Murrah buffalo bull at 18 and 24 months.

The variations in the result between the present study and others may be due to species differences, plane of nutrition, the accuracy of measurements or the calculating method of testicular volume.

The current results for seminal gland length, breadth, thickness and weight appeared to be within the scope of those published by *Osman (1965)* in Egyptian buffalo bulls at 1.5 years and 2.5 years and by *Ghonimi et al. (2014)*. In comparison to the present data, the seminal glands in male buffaloes appeared smaller in size than those of Friesian bulls (*Osman and Zaki, 1965*).

With regard to the fructose concentration in seminal gland tissue, the findings of the current work are almost of those reported by *Eissa (1980)* in buffalo bulls, in which the average values of fructose content per gland at 12 and 16 months of age were 15.18 and 19.55 mg/gland, respectively. However, fructose concentration (mg /gland) showed a gradual insignificant increase with the advance of age in the studied materials. It seems possible that after the age of puberty in buffalo bulls, 2-3 years and more, there is no increase in the seminal gland activity with regard to fructose concentration under the influence of testosterone secretion, which has a similar trend of

variations.

In addition, there is no significant differences in fructose content per gland between different age groups was present. *Fields et al. (1979)* and *Khan et al. (2015)* described that seminal fructose concentrations in young beef bulls were different significantly with ages. However, in a harmony with the obtained results, *Mahmood et al. (2018)* found that the difference in fructose concentration in seminal glands between 18–24 m and  $\geq 24$  was not significant ( $P > 0.05$ ) in buffalo bulls.

A much higher values for fructose concentrations in seminal plasma were reported by *Banerjee and Ganguli (1973)* in Zebu bulls, *Fields et al. (1979)* in Brahman and Montana Hereford bulls. In buffalo bulls, *Barnabe et al. (1993)*; *Ramadan et al. (2009)* and *Andrabi (2014)* reported higher values for fructose content in seminal plasma. This difference was expected as the technique of fructose determination from seminal gland tissue required more steps than those used with seminal plasma.

Testosterone concentration in the present study at 1.5–1.8 years and from 2–2.5 years were slightly higher than those determined by *Ahmad et al. (1984)* at 21 and 25 months, but lower than those at 38 months. Moreover, a lower value was observed by *Hemeida et al. (1985)* in buffalo bulls at 17–19 months of age. In addition, *Sharma, et al. (1984)* reported lower values at 24–30 months of age, but a higher value at 42–48 months were detected. Furthermore, *Mahmood et al. (2018)* reported a lower testosterone values at  $\leq 18$  m and 18–24 months.

With regard to the effect of seasons on the average testosterone levels, it was clear that the highest average testosterone level was noticed during autumn (1.72 $\pm$ 0.61 ng/ml), while the lowest average level was observed during winter season (0.77 $\pm$ 0.34 ng/ml).

*Osman et al. (1983)*; *Javed et al. (2000)* and *Malfatti et al. (2006)* reported a non-significant difference in serum testosterone between different seasons of the year in buffalo bulls. Similar findings were reported by *Sundby and Tollman (1978)* in cattle bull, *Peirce et al. (1987)* in Holstein bulls and *Chacur et al. (2013)* in Simmental bulls. Moreover, *Mahmood et al. (2013)* studied the effect of stress free and stressful seasons on serum testosterone concentration in Cholistani bulls, they found no influence of seasons on them. Similar to the present findings, *Javed et al. (2000)* found that in buffalo bulls more than 11 years old, the higher testosterone levels were found in autumn and humid summer than in winter.

From the obtained results, it is clear that the maximum testosterone value was reported in the summer (August) (4.45 ng /ml), while the minimum level was reported during winter (February) (0.13 ng / ml), which is in agreement with *Perera et al. (1979)* in buffalo bull and *Sundby and Tollman (1978)* in cattle bull. Moreover, *Stumpf et al. (1993)* cited that serum testosterone concentration was highest in the summer (6.18 ng/ml) and lowest in the winter (2.39 ng/ml).

It is clear that, testosterone levels in buffalo bulls vary between different seasons but without significant effect. Therefore, no typical breeding season could be demonstrated in male buffaloes as previously approved by *Osman et al. (1983)*.

## Conclusion

Moreover, the contradictory results on such topics in buffaloes, as appeared from the variable literature, may reflect the absence of selection and unique breeding in such species when compared to cattle raised in developing countries. In addition, the absence of any significant differences in the levels of blood serum testosterone between the different seasons of the year leads to the statement that reproduction in Egyptian water buffalo bull has no typical breeding season.

## Conflict of interest

The authors declare that they have no conflicts of interest.

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