

Theriogenology Dept., Fac. Vet. Med.,  
Tanta University (Kafr El-Sheikh Branch)

**EFFECT OF AGE ON THE SCROTAL  
CIRCUMFERENCE AND TESTICULAR DIMENSIONS  
AND ASSOCIATED SEMEN CHARACTERISTICS AND  
LIBIDO IN BUFFALO BULLS**  
(With 4 Tables)

BY

**I.I EL-KON, and A.A.RAMOUN,**

(Received at 27/9/2003)

تأثير العمر على محيط الصفن ومقاييس الخصية وكذا خصائص السائل  
المنوي والرغبة الجنسية في طلائق الجاموس

اسماعيل اسماعيل القن ، عادل عبد الفتاح رامون

تمت مقارنة كل من مقاييس الخصية (الطول والعرض والسلك) ومحيط الصفن وخصائص السائل المنوي وكذا الرغبة الجنسية في مجموعتين مختلفتين من طلائق الجاموس كل منهما تشمل على خمسة طلائق الاولى تتراوح من ٢٤ الى ٣٠ شهر في العمر ومن ٤٧٥ الى ٥٧٥ كجم في الوزن والثانية تتراوح من ٤٨ الى ٦٠ شهر في العمر ومن ٦٥٠ الى ٧٧٥ كجم في الوزن. كذلك تمت دراسة العلاقة بين هذه المتغيرات وبعضها البعض في المجموعتين الاولى والثانية كل على حده والمقارنة بينهما. اوضحت نتائج هذه الدراسة ان هناك زيادة معنوية في كل من مقاييس الخصية وكذلك محيط الصفن في طلائق المجموعة الثانية مقارنة بطلائق المجموعة الاولى. ايضا في نفس الاتجاه كانت هناك زيادة معنوية في كل من الحركة الجماعية والفردية للحيوانات المنوية وكذلك نسبة الحيوانات المنوية الحية وتركيزها وكذلك العدد الكلي للحيوانات المنوية في القذفة الواحدة بينما كان هناك نقص معنوي في نسبة الحيوانات المنوية المشوهة في طلائق المجموعة الثانية مقارنة بالمجموعة الاولى. بينت الدراسة ايضا انه لم يكن هناك فروق معنوية بين طلائق المجموعة الاولى والثانية بالنسبة لحجم القذفة وكذلك الرغبة الجنسية. سجلت الدراسة الحالية ان هناك علاقة معنوية موجبه بين كل من محيط الصفن ومقاييس الخصية (الطول والعرض والسلك) وكذلك بين أي منهما وكل من الحركة الكلية والفردية للحيوانات المنوية ونسبة الحيوانات المنوية الحية وكذلك تركيزها بينما كانت هناك علاقة معنوية سالبة بين أي من مقاييس محيط الصفن والنسبة الكلية لتشوهات الحيوانات المنوية في طلائق المجموعة الاولى مقارنة بالمجموعة الثانية. اوضحت الدراسة ايضا انه لم تكن هناك علاقة معنوية بين أي من مقاييس الخصية او محيط الصفن وكل من حجم القذفة والرغبة الجنسية في طلائق كل من المجموعة الاولى والثانية على السواء. وقد استنتج من هذه الدراسة انه برغم وجود علاقة طردية بين أي من مقاييس الخصية او محيط الصفن وجودة السائل المنوي في مجموعة الطلائق الاضغر

سنا إلا انه يفضل وضع محيط الصفن في الاعتبار عند اختيار طلائق الجاموس الصغيرة للتبوء بجودة السائل المنوي حيث انه الأسهل والأسرع مقارنة بمقاييس الخصية عند التطبيق الاكلينيكي.

### SUMMARY

Testicular dimensions (T.DS); scrotal circumference (SC), semen characteristics and libido were compared in two groups of buffalo bulls [young (GI) and adult (GII), 5 each]. Also, the correlations between either testicular dimensions (length, width and thickness) and/or scrotal circumference and both libido and semen characteristics were studied. There was a significant ( $P < 0.001$ ) increase in both testicular dimensions and scrotal circumference in GII bulls compared to GI ones. In the same respect the semen quality was better in GII bulls compared to GI ones as was indicated by significant increase in mass motility, individual motility live sperms percentage and sperm cells concentration (at  $P < 0.001$ ) and total sperm count per ejaculate (at  $P < 0.001$ ) and significant decrease in the sperm cells abnormalities (at  $P < 0.01$ ). There was non-significant difference in both ejaculate volume and libido as was expressed by reaction time and number of mountings per ejaculate. Significant ( $P < 0.001$ ) positive correlations were recorded between SC and T.DS including length ( $r = 0.63$ ), width ( $r = 0.55$ ) and thickness ( $r = 0.67$ ) in GI bulls but not in GII ones. Meanwhile there were positive correlations between SC and mass activity ( $r = 0.58$ ,  $P < 0.001$ ); individual motility ( $r = 0.59$ ,  $P < 0.001$ ), live sperm percentage ( $r = 0.39$ ,  $P < 0.01$ ), sperm cells concentration ( $r = 0.47$ ,  $P < 0.001$ ) and negative correlation with sperm cells abnormalities ( $r = -0.73$ ,  $P < 0.001$ ) in GI bulls but not in GII bulls. There were non-significant correlations between SC and total sperms count per ejaculate and libido in both GI and GII bulls. It could be concluded that although there was a direct relationship between either testicular dimensions or scrotal circumference and semen quality in young buffalo bulls, it is preferable to employ the scrotal circumference to predict semen quality during selection among young buffalo bulls as it is rapidly and easily applicable than testicular dimensions.

**Key words:** Buffalo bulls, scrotal circumference, testicular dimensions semen quality.

## INTRODUCTION

During the last three decades, there was an increasing attention towards application of artificial insemination (AI) on large scale in buffalo breeding in Egypt. Such trend necessitates an accurate selection of superior bulls. Selection of sires not only depends on their genotypic and phenotypic attributes but also on the quality of their semen (Rodriguez-Martinez, 1998) as well as on the libido, since poor sexual desire have been identified as a major limiting factors in buffalo breeding (Barnabe *et al.*, 2000). Even though the genetic merit of the bull is superior, its impact is limited by the number of spermatozoa that he produces and this is a direct function of testicular size which is highly correlated to scrotal circumference (Curtis and Amann, 1981). Coulter and Foote (1976) reported that scrotal circumference of the bull is an indirect indication of number and size of seminiferous tubules present in the testes and thereby sperm output. The close relationship between age, body weight and testicular size can possibly be utilized as an indicator to the rate of sperm cell formation especially in young bulls (Hahn, *et al.*, 1969 and Pant *et al.*, 2003).

Scrotal circumference is a useful indicator to predict sperm production potential and may serve as an important criterion for selecting young buffalo bulls as AI sires (Nema and Kodagali, 1994 and Pant *et al.*, 2003) as bulls with higher scrotal circumference produces semen with better quality and spermatozoa with good morphology (Nema and Kodagali, 1994). Although a large number of male calves of high genetic makeup are selected, only few number reach breeding stage because most of them are culled for poor semen quality (Seih *et al.*, 1989). Thus it becomes necessary to establish a norm for predicting both semen quality and libido in buffalo bulls selected to be sires as early as possible. The present study was planned to study.

- 1- Testicular dimensions, scrotal circumference, semen quality and libido in two age groups (young growing and adult mature) of buffalo bulls.
- 2- The correlations between testicular dimensions and/or scrotal circumference and both semen quality and libido in young and adult buffalo bulls.

## MATERIALS and METHODS

### **Bulls and management:**

Ten healthy buffalo bulls kept under the standard management conditions were used in the present study. The animals were assigned on the basis of age into two experimental groups young and adult each of 5 bulls. The bulls in the young group (GI) were 24 to 30 months old and had a body weight range of 475 to 575 kg while those in the adult group (GII) were 48 to 60 months old and had a body weight range of 650 to 775 kg. They were fed on berseem hay and rice straw ad libitum and concentrates that met both maintenance and growing requirements according to NRC (1970).

### **Semen collection and evaluation:**

Semen samples were collected by means of A.V. twice weekly from each bull over a period of 12 weeks extending from the beginning of September till middle of October and the ejaculate was collected directly into a graduated tube and the volume was recorded. Sperm cells concentration and total/sperm count per ejaculate were determined using Neubauer hemocytometer. Both mass and individual motilities were estimated according to Salisbury *et al.* (1978). The percentage of alive sperms was determined in eosin-nigrosin stained films according to Swanson and Bearden (1951). The morphological abnormalities of spermatozoa were determined by the stain smear technique of Salisbury *et al.* (1978).

### **Assessment of libido:**

Libido was assessed by estimation of the reaction time and number of mounting trials per ejaculate.

### **Measuring testicular dimension (T.DS) and scrotal circumference (SC):**

Testicular dimensions (T.DS) including length (cm), width (cm) and thickness (cm) were taken at the points of their maximum dimensions using metal caliper according to Ansari *et al.* (1972). Scrotal circumference (SC) was measured at the largest diameter of the testis using flexible plastic cloth tape according to Almquist *et al.* (1976). Both testicular dimensions (T.DS) and scrotal circumference (SC) were taken once every two weeks.

**Statistical analysis:**

Data were analyzed using t-test and correlations coefficients according to SAS (1985).

**RESULTS**

As has been shown in Table 1, there was a significant ( $P < 0.001$ ) increase in both scrotal circumference and testicular dimensions (length, width and thickness) in adult buffalo bulls (GII) compared to young ones (GI).

Scrotal circumference (SC) was positively significantly ( $P < 0.001$ ) correlated with testicular dimensions viz. length ( $r = 0.63$ ), width ( $r = 0.55$ ) and thickness ( $r = 0.67$ ) in the young buffalo bull (GI) and non-significantly correlated with any of testicular dimensions in case of adult buffalo bulls (GII) (Table, 4).

**Table (1):** Means  $\pm$  standard error (SE) for scrotal circumference and testicular dimensions (length; width and thickness) in young (GI) and adult (GII) buffalo bulls.

Testicular Measurements (cm)	Groups		T-test value
	$\bar{X} \pm SE$		
	Group I	Group II	
Scrotal circumference	29.43 $\pm$ 0.25 b	33.20 $\pm$ 0.20 a	11.60***
Testicular length	6.45 $\pm$ 0.077b	7.31 $\pm$ 0.06 a	8.48***
Testicular width	3.91 $\pm$ 0.04 b	4.74 $\pm$ 0.07 a	9.74***
Testicular thickness	5.25 $\pm$ 0.05 b	6.42 $\pm$ 0.06 a	14.01***

Means in the same raw carrying different letters are significantly different.

\*\*\* Significant at ( $P < 0.001$ ).

The semen quality as indicated by the significant increase in the total sperms count per ejaculate ( $P < 0.01$ ), mass motility, individual motility, live sperm percentage and sperm cells concentration ( $P < 0.001$ ) as well as the significant decrease in the sperm cell abnormalities ( $P < 0.001$ ) was better in the adult (GII) than in the young (GI) buffalo bulls (Table, 2). However there was non-significant variations in the ejaculate volume between adult (GII) and young (GI) bulls (Table, 2).

Table (2): Means  $\pm$  standard error (SE) for semen characteristics (ejaculate volume, mass motility, individual motility, live sperms percentage, sperm cells concentration, sperm cells abnormalities, and total sperms count per ejaculate in young (GI) and adult (GII) buffalo bulls.

Parameter	Groups		T-test value
	$\bar{X} \pm SE$		
	Group I	Group II	
Ejaculate volume (ml)	3.88 $\pm$ 0.20 a	3.57 $\pm$ 0.17a	1.17 ns
Mass motility	2.65 $\pm$ 0.074 b	3.85 $\pm$ 0.057 a	12.76***
Individual motility %	67.91 $\pm$ 0.88 b	80.16 $\pm$ 0.60a	11.43***
Live sperms %	89.45 $\pm$ 0.58 b	93.60 $\pm$ 0.36 a	5.99***
Sperm cells concentration ( $n \times 10^6$ )	707.03 $\pm$ 25.94 b	987.65 $\pm$ 19.07 a	8.71***
Total sperm count per ejaculate ( $n \times 10^8$ )	2.77 $\pm$ 0.19 b	3.58 $\pm$ 0.20 a	2.94**
Sperm cells abnormalities %	9.38 $\pm$ 0.33 a	3.40 $\pm$ 0.15 b	16.34***

Means in the same row carrying different letters are significantly different.

NS = Non significant

\*\* Significant at ( $P < 0.01$ ).

\*\*\* Significant at ( $P < 0.001$ ).

The correlations between either of scrotal circumference (SC) or testicular dimensions vz: length, width and thickness and semen characteristic were summarized in Table, 4.

There were significant positive correlations between scrotal circumference and each of mass motility, individual motility percentage and sperm cell concentration at  $P < 0.001$  and live sperm percentage at  $P < 0.01$  while there were significant negative correlations between SC and ejaculate volume at  $P < 0.01$  and sperm cell abnormalities at  $P < 0.001$  in GI bulls. However there was non-significant correlation between SC and total sperm count per ejaculate in GI bulls. In contrast, there were non-significant correlations between SC and any of the studied semen characteristics in the present study in GII bulls (Table, 4). Concerning the correlations between T.DS and various semen characteristics the results of the present study revealed that while there were significant positive correlations between testicular length and mass and individual motilities at  $P < 0.001$  and live sperm percentage and sperm cell concentration at  $P < 0.01$  in GI bulls, there were non-significant correlations between testicular length and each of mass motility, individual motility and live sperm percentage in GII bulls. Moreover, significant ( $P < 0.01$ ) negative correlation was recorded between testicular length and sperm cell concentration in GII bulls. A significant ( $P < 0.01$ ) negative correlation was recorded between testicular length and ejaculate volume in both GI and GII bulls as well as

between testicular length and sperm cell abnormalities in GI bulls only ( $P < 0.001$ ). However, non-significant correlations were recorded between testicular length and total sperm count per ejaculate in both GI and GII bulls.

Regarding the correlations between testicular width and various semen characteristics, the present study showed that there were significant positive correlations between testicular width and mass motility, individual motility and sperm cell concentration at  $P < 0.001$  as well as live sperm percentage at  $P < 0.01$  in GI bulls. On the other hand there were non-significant correlations between testicular width and each of mass motility, live sperm (%) and sperm cell abnormalities in GII bulls. Significant ( $P < 0.05$ ) negative correlations were recorded between testicular width and both individual motility and sperm cell concentration in GII bulls. Nevertheless significant ( $P < 0.001$ ) negative correlation was recorded between testicular width and sperm cell abnormalities in GI bulls, non-significant correlation was recorded between testicular width and sperm cell abnormalities in GII bulls. However, non-significant correlations were recorded between testicular width and both of ejaculate volume and total sperm count per ejaculate in both GI and GII bulls.

Nevertheless there were significant positive correlation between testicular thickness and mass motility, live sperm % and sperm cell concentration at  $P < 0.01$  and individual motility at  $P < 0.01$  in GI bulls, non-significant correlations were recorded between testicular width and any of these parameters in GII bulls. Significant negative correlations were recorded between testicular width and sperm cell abnormalities in GI ( $P < 0.001$ ) and GII ( $P < 0.01$ ) bulls. Significant ( $P < 0.05$ ) negative and non-significant correlations were recorded between testicular thickness and ejaculate volume in GI and GII bulls respectively. However, non-significant correlations were recorded between testicular thickness and total sperm count per ejaculate in both GI and GII bulls.

The sexual desire as indicated by the reaction time and number of mounting trials per ejaculate did not differ significantly between young bulls (GI) and adult ones (GII) (Table, 3). Non significant correlations were recorded between each of scrotal circumference, testicular: Length, width and thickness and either of reaction time or number of mounting trials per ejaculate in both young (GI) and adult (GII) bulls (Table 4).

**Table (3):** Means  $\pm$  standard error (SE) for sexual behaviour as indicated by the reaction time and number of mountings trials per ejaculate in young (GI) and adult (GII) buffalo bulls.

Sexual behaviour	Groups		T-test value
	$\bar{X} \pm SE$		
	Group I	Group II	
Reaction time (sec.)	49.91 $\pm$ 3.08 a	48.73 $\pm$ 2.21 a	0.312 ns
Number of mounting trials per ejaculate	1.90 $\pm$ 0.06 a	1.96 $\pm$ 0.007 a	0.70 ns

**Table (4):** The correlations between either of scrotal circumference or testicular dimensions (length, width and thickness) and sexual desire indicated by reaction time and number of mounting per ejaculate and various semen characteristics in young (GI) and adult (GII) buffalo bulls.

Character	Scrotal circumference		Testicular dimensions					
			Testicular length		Testicular width		Testicular thickness	
	GI	(GII)	GI	GII	GI	GII	GI	GII
Scrotal circumference	1.00	1.00	0.63***	0.255 ns	0.55***	-0.067 ns	0.67***	0.039 ns
Reaction time (sec)	0.11 ns	0.129 ns	-0.002 ns	-0.056 ns	-0.09 ns	-0.273*	0.01 ns	-0.73 ns
Number of mountings per ejaculate	0.14 ns	0.006 ns	0.13 ns	0.196 ns	0.04 ns	-0.187 ns	0.09 ns	-0.038 ns
Ejaculate volume (ml)	-0.32**	-0.269 ns	-0.23***	-0.34**	-0.06 ns	0.002 ns	-0.15*	-0.061 ns
Mass motility (No)	0.58***	0.111 ns	0.45***	-0.076 ns	0.43***	-0.150 ns	0.588***	0.030 ns
Individual motility %	0.59***	0.107 ns	0.46***	-0.007 ns	0.37***	-0.281*	0.55**	0.037 ns
Live sperm %	0.39**	-0.155 ns	0.24**	-0.14 ns	0.23**	-0.042 ns	0.36***	-0.007 ns
Sperm cells concentration (n x 10 <sup>6</sup> )	0.47***	-0.147 ns	0.33**	-0.34**	0.44***	-0.215*	0.53***	0.107 ns
Total sperm count per ejaculate (n x 10 <sup>9</sup> )	0.05 ns	0.0278 ns	0.02 ns	0.42 ns	0.018 ns	0.098 ns	0.14 ns	0.009 ns
Sperm cells abnormalities	-0.73***	-0.187 ns	-0.48***	-0.017 ns	-0.51***	0.042 ns	-0.70***	-0.277*

ns = non-significant  
\*\* = significant (P < 0.01)

\* = significant (P < 0.05)  
\*\*\* = significant (P < 0.001)



## DISCUSSION

Although large number of male calves of high genetic merit are selected, only few actually reach breeding stage because most of them are culled for poor semen quality and/or growth (Seth *et al.*, 1989). Thus it becomes essential to establish basis for predicting the quality of semen as early as possible. As has been shown by the results of the present study there was a significant increase in both testicular dimension (T.DS) and scrotal circumference (SC) in the adult buffalo bulls compared to younger ones. Meanwhile the semen quality was more better in adult bulls than in younger ones as well. These results coincided with Heuer and Bajwa (1986) and Younis *et al.* (2003) who reported that scrotal circumference was higher in adult buffalo bulls than in younger ones. Also, Sundararaman *et al.* (2002) reported that testes size increases with age of bulls until it reaches the maximum growth at > 60 to 70 months and thereafter a moderate decline in the testes size is observed.

As has been proved by the results of the present study the comparison between young and adult buffalo bulls in relation to T.DS, SC and various semen characteristics revealed presence of positive correlation between age and each of SC, T.DS (Brito *et al.*, 2002) and various semen characteristics (Nema and Kodagali, 1994) a finding which suggest presence of positive correlation between scrotal circumference and semen quality (Brinks *et al.*, 1978). However, there were significant positive correlations between either of SC or T.DS (length, width and thickness) and mass motility, individual motility, live sperm percentage and sperm cells concentration and significant negative correlations with ejaculate volume and sperm cell abnormalities in younger bulls but not in older ones (Table, 4). These results may be attributed to the fact that the testis in younger bulls exhibit a parallel increase in both size (Veerapandian, 1992 and Sundararaman *et al.*, 2002) and spermatogenic activity (Kumi-Diaka *et al.*, 1981) until sexual maturity is attained thereafter the semen characteristics remain relatively stable until the age of senility where they begin to decline, an explanation which may coincide with that suggested by El-Azab *et al.* (1977) and McCool and Entwistle (1989). In the same respect Luthra *et al.* (1993) reported that the increase in SC was correlated positively with sperm motility and negatively with the total sperm abnormalities in growing bulls but as maturity is reached other factors such as stress and infection are likely to be of great importance than age (Salisbury *et al.*, 1978). Moreover Carter

*et al.* (1980) concluded that sperm production was positively correlated to testis size in young animals and decline with age. In contrast to previous studies (El-Azab *et al.*, 1977 and Luthra *et al.*, 1993) which recorded presence of significant positive correlation between scrotal circumference and ejaculate volume, the results of the present study revealed a non-significant or even negative significant correlations between either T.DS or SC and ejaculate volume in both young and adult buffalo bulls. However, such result may coincide with Sundarraraman *et al.* (2002) who recorded non-significant correlation between testicular size and ejaculate volume. The confliction between the results of the present study and previous ones was clarified by the finding of White (1975) who concluded that the variation in ejaculate volume could be mainly due to differences in the secretory activity of sex glands.

Concerning the correlation between SC and libido, the results of the present study showed non significant correlation as expressed by both reaction time and number of mounting per ejaculate in both young and adult bulls. However, such results may come in accordance with Mansour *et al.* (1989) who found no correlation between sexual desire and either of age or scrotal circumference in buffalo bulls.

It could be concluded that although there was a significant positive correlation between either of testicular dimensions or scrotal circumference and semen quality in young buffalo bulls, it is preferable to employ the scrotal circumference to predict semen quality during selection of young buffalo bulls as it is easily and rapidly applicable than measuring testicular dimension.

#### REFERENCES

- Almqvist, J.O.; Branas, R.J. and Barber, K.A. (1976):* Post pubertal changes in semen production of Charolais bulls ejaculated at high frequency and the relation between testicular measurements and sperm output. *J. Anim. Sci.* 42, 670.
- Ansari, M.M.; Thomas, B.P.; Shukla, R.K. and Menon, G.N. (1972):* Association of testicular measurements with semen production in surti buffaloes bulls. *Ind. J. Anim. Sci.* 42: 465-467.
- Barnabe, V.H.; Molero-Filho, J.R.; Baruselli, P.S. and Barnabe, R.C. (2000):* Modified serving capacity test (MSCT) in Murrah buffalo bulls (*Bubalus bubalis*). *Buffalo, J.* 2, 231-236.

- Brinks, J.S.; Mcinerney, M.J. and Cheornweth, P.J. (1978):* Relationship of age at puberty in heifers to reproductive traits in young bulls. *Proc. West. Sec. Amer. Soc., Anim. Sci.*, 29: 28.
- Brito, L.F.; Silva, A.E.; Rodrigues, L.H.; Vieira, F.V.; Deragon, L.A. and Kastelic, J.P. (2002):* Effect of age and genetic group on characteristics of the scrotum, testis and testicular vascular cones and on sperm production and semen quality in AI bulls in Brazil. *Theriogenol.*, 58(6): 1175-1186.
- Carter, A.P.; Wood, P.D.P. and Penelope, A. Wright (1980):* Association between scrotal circumference, live weight and sperm output in cattle. *J. Reprod. Fertil.*, 59, 447-451.
- Coulter, G.H. and Foote, R.H. (1976):* Relationship of testicular weight to age and scrotal circumference of Holstein bulls. *J. Dairy Sci.*, 59: 730-732.
- Curtis, S.K. and Amann, R.P. (1981):* Testicular development establishment of spermatogenesis in Holstein bulls. *J. Anim. Sci.*, 53(6): 1645-1657.
- El-Azab, A.I.; Rakha, A.M.; Zaki, K. and Elchahidi, A.A. (1977):* Studies of some factors affecting semen production in buffalo bulls. *Egypt. J. Vet. Sci.*, 14(2): 115-123.
- Hahn, W.R.; Foote, R.H. and Seidel, J.R. (1969):* Testicular growth and related sperm output in dairy bulls. *J. Anim. Sci.*, 29, 41-46.
- Heuer, C. and Bajwa, M.A. (1986):* Selection of Nili-Ravi buffaloes for artificial insemination. *Zuchthygiene*, 21(5): 257-262.
- Kumi-Diaka, J.; Nagaratam, V. and Rwuuan, J. (1981):* Seasonal and age related changes in semen quality and testicular morphology of bulls in a tropical environment. *Vet. Rec.* 108, 13-15.
- Luthra, R.; Maridov, M.F. and Georgievs, S. (1993):* Scrotal circumference in relation to seminal characteristics of young growing bulls. *Ind. J. Anim. Sci.*, 63(6): 641-643.
- Mansour, H. Hazzaa, A.; Abdel-Raheim, A. and Eidaroos, A. (1989):* reproductive capacity of buffalo bulls. 1. Scrotal measurements and their relation to age libido and semen characteristics in sexually mature buffalo bulls. *ESARF, First Annual Congress, Edfina, January, 24-25, 1989.*
- McCool, C.J. and Entwistle, K.W. (1989):* Reproduction function in the Australian swamp buffalo bull: age effects and seasonal effects. *Theriogenol.*, 31(3): 583-594.

- Nema, S.P. and Kodagali, S.B. (1994):* Trans-scrotal circumference (TSC), Age, body weight and seminal characters in Surti buffaloes. *Ind. J. Anim. Reprod.* 15(2): 154-156.
- NRC (1970):* Nutrient requirements of domestic animals No. 4, Nutrient Requirements of beef cattle; National Research Council Washington DC.
- Pant, H.C.; Sharma, R.K.; Patel, S.H.; Shukla, H.R.; Mittal, A.K.; Kasiraj, R.; Misra, A.K. and Prabhakar, J.H. (2003):* Testicular development and its relationship to semen production in Murrah buffalo bulls. *Theriogenol.*, 60(1): 27-34.
- Rodriguez-Martinez, H. (1998):* Optimization of sperm quality in AI bulls. *Rep. Dom. Anim.*, 33, 233-237.
- Salisbury, G.W.; Vandemark, N.L. and Lodge, J.R. (1978):* Physiology of reproduction and artificial insemination of cattle. 2 ed. W.H. Freeman and Co. San, Francisco, USA.
- SAS (1985). SAS User's Guide: Statistics. SAS Inst., Inc. Cary, N.C.*
- Seth, R.K.; Raina, V.S.; Joshi, B.K. and Gurnan, M. (1989):* Multistage section of crossbred males and effect of their age and body weight on semen quality and freezeability. *Ind. J. Anim. Sci.* 59(1): 171-174.
- Sundararaman, M.N.; Thangaraju, P. and John-Edwin, M. (2002):* Age related changes in testis size of Jersey bulls and its effect on semen production traits. *Ind. J. Anim. Sci.*, 72(7): 567-568.
- Swanson, E.W. and Bearden, H.J. (1951):* An eosin-Nigrosin stain for differentiating of live dead bovine sperms. *J. Anim. Sci.*, 10(4): 981.
- Veerapandian, C. (1992):* Breeding soundness examination and hormone profile in breeding bulls Ph.D. Thesis submitted to the Tamil Nadu Veterinary and Animal Sciences University Channel 600 007-
- White, J.C. (1975):* Mammalian semen reproduction in farm animals in: *Reproduction in Farm Animals* (Ed) Hafez E.S.E. 3<sup>rd</sup> ed., p. 101-122. Lea & Febiger Philadelphia, U.S.A.
- Younis, M.; Samad, H.A.; Ahmad, N. and Ahmad, I. (2003):* Effect of age and season on the body weight, scrotal circumference and libido in Nili-Ravi buffalo bulls maintained at the semen production unit, Qadirabd. *Pakist. Vet. J.* 23(2), 59-65.