



Relationship of Age, Breed and Libido with Semen Traits of Cattle Bulls

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ABSTRACT

The present study was conducted to determine correlation of semen quality with libido in different breeds of bulls. Four mature cattle bulls from each of Friesian, Jersey, Achai, Cross (Friesian x Sahiwal) and Sahiwal in different age (1-3, 3-5 and above 5 years) were selected. For age grouping, data of the entire breed was pooled. Libido was measured in terms of time (seconds) taken for the entering arena until mounting (P1), time (seconds) taken from mounting the teaser until erection (P2) and reaction time (seconds) (P3). Semen was collected twice a week and analyzed for semen volume, sperm concentration, sperm motility, dead sperm percentage and hyposmotic swelling test (membrane integrity). The results revealed that libido score in term of P1 (6.6 ± 0.46 Sec), P2 (1.88 ± 0.07 Sec) and P3 (9.57 ± 0.49 Sec) was significantly high ($P < 0.05$) in Jersey breed, while crossbred bulls showed the lowest libido score. Jersey bulls showed significantly high ($P < 0.05$) semen characteristics including sperm concentration ($1560.0 \pm 59.00 \times 10^6/\text{ml}$), membrane integrity ($77.42 \pm 0.97\%$) and livability ($83.32 \pm 8.32\%$). Libido calculated at different age groups showed that older animals having age above five years exhibited the best libido (P1, P2 and P3) compared to the younger animals. Similarly, semen characteristics including semen volume (6.50 ± 0.73 ml) and membrane integrity ($74.00 \pm 1.40\%$) were significantly high ($P < 0.05$) in older bulls (above 5 years) compared to the young animals with no significant effect ($P > 0.05$) on sperm concentration, motility and dead sperm percentage. Membrane integrity showed significantly high positive correlation with sperm concentration ($r = 0.52$) and significantly low negative correlation with dead sperm percentage ($r = -0.28$). We concluded from the results that Jersey breed showed higher libido score and semen quality. Libido and semen quality were higher in Jersey bulls compared to the other breeds. Moreover, libido and semen quality increased with increasing age (up to 8 years).

Article Information

Received 23 May 2015

Revised 10 February 2016

Accepted 25 May 2016

Available online 25 September 2016

Authors' Contribution

MSQ designed the study. HR carried out experimental work and compiled the data. MSQ and RUK analyzed the data. RUK wrote the article. All others helped in finalizing the manuscript.

Key words

Cattle bulls,
Semen quality,
Libido, Age, Breed

INTRODUCTION

One of the preconditions of artificial insemination (AI) is that sire should be supposed to have very high reproductive performance such as sexual activity and ejaculatory performance (Rehman *et al.*, 2014). These factors mainly depend upon breed, age, genetic, libido and environment (Ahmad *et al.*, 2003; Petherick, 2005; Galina *et al.*, 2007; Beran *et al.*, 2011; Kondracki *et al.*, 2013). The level of sexual excitement and performance can affect the ejaculatory performance and semen quality (Pound *et al.*, 2002; Levis and Reicks, 2005; Kondracki *et al.*, 2013). The reproductive competence of commercial herds is on the edge of decline in the last few decades. The reasons have been sought by investigating female fertility; however, little

attention has been paid to the contribution of male in overall fall in fertility of the herd (Beran *et al.*, 2011).

Libido has been referred to sexual motivation, discovered through behaviors such as looking for mating, detection of estrus, courtship, and mating (Parkinson and Vermunt, 2000). Libido is typically calculated by means of the reaction time, defined as the elapsed time between exposure to stimuli and first service (Ott and Memon, 1980). Libido is the desire and excitement of a male animal to mount and do matting with a female animal (Chenoweth, 1981). Libido is a helpful parameter of reproductive competence (Ahmad *et al.*, 2005). For a successful AI program, animals showing high libido and appropriate mating capability are desirable characteristics (Chenoweth, 1983; Ahmad *et al.*, 2005). Bulls with high libido can produce satisfactorily higher number of viable spermatozoa through multiple ejaculates in a relatively short time (Ahmad *et al.*, 2005). Pregnancy rates, time of conception, length of calving seasons, and homogeneity of calves at weaning can be improved with the selection

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0030-9923/2016/0006-1793 \$ 8.00/0
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of bulls with better libido (Blockey, 1978; Chenoweth, 1981; Ahamd *et al.*, 2005).

Examination of a bull in a controlled area with one or more than one females in estrus is the simplest and easiest method of evaluating the libido of the breeding bull used for the artificial insemination program (Chenoweth, 1983; Kondracki *et al.*, 2013). In our country, the criteria for the selection of bull comprises the milk yield of the dam, phenotypic characteristics, winning a national or provincial competition and evaluation of semen characteristics (Ahmad *et al.*, 2003; Khan *et al.*, 2007). The criteria adopted to select a bull of enough reproductive ability are seen in latest areas by introducing modern laborious techniques. Assessment of libido, however, is still a reliable technique to assess the soundness of a bull for selection of breeding program (Mahmood *et al.*, 2014). Testing animals on the basis of libido could provide an easy assessment to select a bull for breeding program. In the previous reports, the libido has largely been scored on the basis of grading (Ahmad *et al.*, 2005; Younis *et al.*, 2003) which is estimated on the basis of visual observation and may give false results. Therefore, in the present study, we designed a research plan to score the libido on the basis of timing interval between different stages of copulation as described recently by Kondracki *et al.* (2013).

The specific objectives of this study were to find the libido of bulls in each breed at different age and their correlation with semen characteristics.

MATERIALS AND METHODS

Four healthy and mature bulls each from Jersey, Friesian, Sahiwal, Achai and crossbred (Sahiwal and Friesian) were selected at Harichand, Charsadda, Pakistan. The animals were further categorized according to different age groups. Group I: up to 3 years, group II: 3-5 years and group III: above five years as suggested by Ahmad *et al.* (2003). Semen was collected twice a week (Monday and Thursday). The libido of each bull was calculated by measuring the following parameters as described by Kondracki *et al.* (2013) with slight modifications. (1) Time (seconds) taken for entering the arena until mounting; (2) Time (seconds) from mounting the teaser until developing of erection; (3) Reaction time (seconds), and (4) Number (n) of times a bull mounted to yield semen.

Semen was collected twice a week from each bull of each breed using artificial vagina. Semen quality parameters were determined as described by Qureshi (2011). Semen volume was measured with the help of graduated tube. Semen was immediately transferred to a water bath having temperature 37°C. Sperm

concentration was measured with the help of haemocytometer. The percentage of motile sperm and dead spermatozoa was performed as described by Qureshi (2011). Hypo-osmotic swelling test (HOST) was conducted by mixing 0.1 ml of the semen with 1 ml of test solution [0.5 ml of D-fructose (1.47%) and sodium citrate 2.7%]. The mixture was incubated at 37°C for 40 minutes. The spermatozoa showing coiled and swollen tails were considered positive for HOST.

Data analysis

Data obtained was analyzed using one way analysis of variance (ANOVA). Statistical difference between means was calculated using Duncan Multiple Range Test (Duncan, 1955). Pearson's correlation was used to find the correlation between the semen traits and libido parameters.

RESULTS AND DISCUSSION

The present study was investigated to find the effect of libido, age and breed on semen quality and their inter relationship. Under most of the circumstances, the bulls for the purpose of AI are selected on the data obtained from physical and andrological parameters excluding the libido. In the current research work, libido of bulls was also taken into account. The results of libido parameters in different breeds are shown in Table I.

Table I.- Libido parameters in different breeds of cattle bull.

Para- meters	Friesian	Jersey	Achai	Cross	Sahiwal
P1	13.26± 0.93 ^b	6.68± 0.46 ^c	15.37± 1.37 ^b	29.89± 4.78 ^a	11.51± 4.54 ^{bc}
P2	2.47± 0.13 ^{ab}	1.88± 0.07 ^b	2.08± 0.24 ^{ab}	3.79± 1.56 ^a	1.92± 0.38 ^{ab}
P3	17.44± 0.95 ^b	9.57± 0.49 ^c	19.39± 1.43 ^b	34.50± 4.82 ^a	14.78± 4.72 ^{bc}
P4	4.09± 0.10 ^a	4.97± 0.12 ^a	3.42± 0.19 ^b	3.29± 0.23 ^b	3.66± 0.33 ^b

^{ac} Mean value within a row with different superscripts differ significantly (P<0.05). P1, Time (seconds) taken for the entering the arena until mounting; P2, Time (seconds) from mounting the teaser until developing of erection; P3, Reaction time (seconds); P4, Number (n) of times a bull mounted to yield semen.

The result showed that time (Sec) from entering the arena until mounting was significantly (P<0.05) low in Jersey and significantly (P<0.05) high in Crossbred. Time (Sec) from mounting the teaser until developing of erection was significantly (P<0.05) low in Jersey and significantly (P<0.05) high in Crossbred. Reaction time

(Sec) was significantly ($P < 0.05$) low in Jersey and significantly ($P < 0.05$) high in Crossbred. Number (n) of times a bull mounted to yield semen was significantly ($P < 0.05$) high in Jersey and Friesian and significantly ($P < 0.05$) low in Achai, Crossbred and Sahiwal bulls. In the current study, we found that breed and age significantly affected the libido and semen quality in cattle bulls. In the past, different authors reported varying level of libido in farm animals (Rhen and Crews, 2002; Younis *et al.*, 2003; Ahmad *et al.*, 2005; Kondracki *et al.*, 2013). Libido measurement is a useful tool to judge reproductive efficiency in a bull. Ahmad *et al.* (2005) concluded that a bull exhibiting higher libido may carry better semen quality.

The results of libido parameters in different breeds are shown in Table II. The result showed that semen volume was not significantly ($P > 0.05$) affected in the breeds studied in this work. The sperm concentration was significantly ($P < 0.05$) high in Jersey and was significantly ($P < 0.05$) low in Friesian, Achai, Cross and Sahiwal cattle bulls. Sperm motility was not significant ($P > 0.05$) in all breeds. Dead sperm percentage was significantly ($P < 0.05$) high in Crossbred and was significantly ($P < 0.05$) low in Friesian, Jersey, Achai and Sahiwal. Membrane integrity (HOST) was significantly ($P < 0.05$) high in Jersey and Sahiwal cattle bulls and significantly ($P < 0.05$) low in Crossbred animals. In the present study, it was observed that semen characteristics vary between the breeds. Our results are supported by the findings of Andrabi *et al.* (2002) who reported that semen characteristics are affected by a number of factors including the breed. In the current study, it was observed that libido was significantly high in Jersey compared to the other breeds. In the same line, the semen quality in term of sperm concentration and membrane integrity was significantly higher in Jersey bulls. In line of our results, Ahmad *et al.* (2005) concluded that a bull with higher libido manifests higher sperm concentration, multiple ejaculates and fertility.

The results of libido parameters in different age groups are shown in Table III. The result showed that time (Sec) the entering the arena until mounting was significantly ($P < 0.05$) low in above 5 year animals and significantly ($P < 0.05$) high in 1-3 years and 3-5 years animals. Time (Sec) from mounting the teaser until developing of erection was non-significant ($P > 0.05$) in all age groups. Reaction time (Sec) was significantly ($P < 0.05$) low in above 5 years animals and significantly ($P < 0.05$) high in 1-3 years and 3-5 years animals. Number (n) of times a bull mounted to yield semen was significantly ($P < 0.05$) high in 1-3 years animals and significantly ($P < 0.05$) low in above 5 years animals. In the past, different authors reported a relationship of age

with the semen quality. Younis *et al.* (2003) and Ahmad *et al.* (2005) reported that libido of older bull was higher than the younger Nilli-Ravi bulls and Sahiwal bulls respectively which is in line with our results. The older bulls are exposed to repeated experience of semen collection; therefore, they become habitual to the process and take little time during semen collection. Rhen and Crews (2002) concluded that sexual skill and copulatory experience improve with the age. According to Petherick (2005) libido expression is greater in older bull and varies with the genetic of the bull.

Table II.- Semen volume, sperm concentration, motility, dead sperms and HOST in different breed of cattle bulls.

Parameters	Friesian	Jersey	Achai	Cross	Sahiwal
Volume (ml)	6.82± 0.56 ^a	6.42± 0.69 ^a	6.00± 0.43 ^a	7.30± 0.36 ^a	5.50± 0.04 ^a
Concentration (× 10 ⁶)	1351.7± 24.50 ^b	1560.0± 59.00 ^a	1333.3± 62.20 ^b	1315.8± 30.27 ^b	1300.0± 131.94 ^b
Motility (%)	77.51± 0.65 ^a	76.94± 0.81 ^a	78.88± 1.67 ^a	80.90± 1.58 ^a	76.50± 3.55 ^a
Dead (%)	18.32± 0.47 ^b	17.68± 0.58 ^b	17.55± 1.19 ^b	25.80± 1.13 ^a	19.00± 2.54 ^b
HOST (%)	71.69± 0.79 ^{ab}	77.42± 0.97 ^a	72.44± 2.00 ^{ab}	68.20± 1.90 ^b	74.50± 4.25 ^a

^{ab} Mean value within a row with different superscripts differ significantly ($P < 0.05$)

HOST: hyposmotic swelling test

Table III.- Libido parameters in different breeds of cattle bull according to their age.

Age groups	P1	P2	P3	P4
1-3 years	13.50± 1.66 ^a	2.51± 0.36 ^a	17.53± 1.72 ^a	3.24± 0.16 ^a
3-5 years	13.50± 1.12 ^a	2.31± 0.24 ^a	17.19± 1.16 ^a	3.12± 0.10 ^{ab}
Above 5 years	11.74± 1.34 ^b	2.15± 0.29 ^a	15.49± 1.38 ^b	2.87± 0.12 ^b

For statistical details and abbreviations, see Table I.

The results of semen evaluation in different age groups are shown in Table V. The result showed that semen volume was significantly ($P < 0.05$) high in 3-5 years and above 5 years of animals and significantly ($P < 0.05$) lower in 1-3 years animal age. There was no significant difference in sperm concentration, motility and dead percentage in all age groups. Membrane integrity was significantly ($P < 0.05$) high in above 5 years animal and significantly ($P < 0.05$) lower in 1-3 years of

Table IV.- Pearson's correlation between physical and libido parameters.

	P1	P2	P3	P4	Host	Concentration	Dead	Motility
P2	0.260							
	0.004							
P3	0.984	0.305						
	0.000	0.000						
P4	-0.044	-0.056	-0.019					
	0.637	0.546	0.833					
Host	-0.071	-0.056	-0.064	0.053				
	0.445	0.545	0.493	0.568				
Concentration	0.078	0.090	0.076	-0.040	0.223			
	0.400	0.334	0.413	0.663	0.015			
Dead	-0.089	-0.162	-0.077	0.100	-0.272	0.198		
	0.336	0.080	0.405	0.282	0.003	0.032		
Motility	0.067	0.062	0.067	0.093	0.247	0.524	-0.288	
	0.470	0.503	0.468	0.316	0.007	0.000	0.001	
Volume	0.080	-0.104	0.083	0.128	0.125	-0.178	0.176	0.065
	0.038	0.261	0.369	0.168	0.178	0.053	0.057	0.480

For statistical details and abbreviations, see Table I.

Table V.- Semen volume, concentration, motility, dead sperms and HOST in different breeds of cattle bull according to age groups.

Age Groups	Volume (ml)	Concentration ($\times 10^6$)	Motility (%)	Dead (%)	HOST (%)
1-3 years	5.78 \pm 0.72 ^b	1373.9 \pm 42.27 ^a	77.04 \pm 1.20 ^a	17.21 \pm 0.54 ^a	69.97 \pm 0.94 ^b
3-5 years	6.19 \pm 0.68 ^a	1369.2 \pm 27.67 ^a	78.48 \pm 0.76 ^a	19.04 \pm 0.69 ^a	72.38 \pm 0.85 ^{ab}
Above 5 years	6.50 \pm 0.73 ^a	1336.8 \pm 31.19 ^a	78.00 \pm 0.70 ^a	18.50 \pm 0.54 ^a	74.00 \pm 1.40 ^a

^{ab} Mean value with different superscripts differ significantly $P < 0.05$. HOST: hyposmotic swelling test

animals age. In an earlier report, a relationship was observed between the semen quality and age of the bull (Padrik *et al.*, 2012). According to Sajjad *et al.* (2007) sexual performance starts when a bull reached the age of puberty and maintains it for long time before reaching old age. Furthermore, best semen characteristics are found during the age of 3-4 years. Javed *et al.* (2000) found a trend of increasing semen volume in buffalo bulls with advancing age (up to 12-15 years old). Similar observations were also reported by some other researchers in the past (Nordin *et al.*, 1990; Younis, 1996). Mahmood *et al.* (2014) found a significant positive correlation between age and semen volume and significant negative relationship with the sperm concentration and sperm viability. Previous reports indicate that semen characteristics improved with the advancing age and then declined after a specific age due to senile atrophy (Khan *et al.*, 2007; Bhakat *et al.*, 2011).

The relationship of physical parameters of semen and libido parameters are shown in Table V. The result revealed that P1 has significantly ($P < 0.05$) positive correlation with P2, P3 and semen volume. Similarly, P2

has significant ($P < 0.05$) positive correlation with P3. Membrane integrity showed significantly ($P < 0.05$) high positive correlation with sperm concentration and significantly ($P < 0.05$) low negative correlation with dead sperm percentage. Similarly, sperm concentration had significantly ($P < 0.05$) high positive correlation with dead sperm percentage and motility. In addition, dead sperm percentage exhibited significant ($P < 0.05$) negative correlation with sperm motility. Previous studies have demonstrated a positive correlation of membrane integrity and sperm motility (Mandal *et al.*, 2003; Padrik *et al.*, 2012) or negative correlation with DNA damage (Stranger *et al.*, 2010). Our result agreed with the findings of Javed *et al.* (2000) who reported positive correlation between sperm concentration and mass motility and progressive motility.

CONCLUSION

Jersey breed showed higher libido and semen quality. Libido and semen quality are increasing with increasing age (up to 8 years). Pearson's co-relation

showed that HOST and sperm concentration, semen volume and sperm concentration, HOST and motility as well as libido and semen volume have positive correlation. Negative correlation was found between HOST and dead sperm percentage; sperm concentration and semen volume. The present study concluded that semen quality of the cross bred bulls should be improved to reduce reproductive fertility problems. Libido parameters should also be tested in other domestic animal and relationship between libido and post-thawed semen should be explored.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group No RG-1436-021.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

- Ahmad, M., Asmat, M.T. and Najib-ur-Rehman. 2005. Relationship of testicular size and libido to age and season in Sahiwal bulls. *Pak. Vet. J.*, **25**: 67-70.
- Ahmad, M., Asmat, M.T., Najib-ur-Rehman and Khan, M.Z., 2003. Semen characteristics of Sahiwal bulls in relation to age and season. *Pak. Vet. J.*, **23**: 202-206.
- Andrabi, S.M.H., Naheed, S., Khan, L.A. and Ullah, N., 2002. Semen characteristics of crossbred (Friesian x Sahiwal) and Sahiwal young bulls maintained under subtropical conditions of Punjab. *Pak. Vet. J.*, **23**: 100-102.
- Beran, J., Stádník, L., Ducháček, J., Toušová, R., Louda, F. and Štolc, L., 2011. Effect of bulls' breed, age and body condition score on quantitative and qualitative traits of their semen. *Acta Univ. Agric. Silvicul. Mendel. Brunen.*, **59**: 37-44.
- Bhakat, M., Mohanty, T.K., Raina, V.S., Gupta, A.K., Khan, H.M., Mahapatra, R.K. and Sarkar, M., 2011. Effect of age and season on semen quality parameters in Sahiwal bulls. *Trop. Anim. Hlth Prod.*, **43**: 1161-1168.
- Blockey, M.A., 1978. The influence of serving capacity of bulls on herd fertility. *J. Anim. Sci.*, **46**: 589-595.
- Chenoweth, P.J., 1981. Libido and mating behavior in bulls, boars and rams. A review *Theriogenology*, **16**: 155-177.
- Chenoweth, P.J., 1983. Sexual behavior of the bull. A review. *J. Dairy Sci.*, **66**: 173-179.
- Galina, C.S., Horn, M.M. and Molina, R., 2007. Reproductive behavior in bulls raised under tropical and subtropical conditions. *Horm. Behav.*, **52**: 26-31.
- Javed, M.T., Khan, A. and Kausar, R., 2000. Effect of age and season on some semen parameters of Nili- Ravi buffalo (*Bubalus bubalis*) bulls. *Vet. Arhiv.*, **70**: 83- 94.
- Khan, M.S., Bhatti, A.A., Bhatti, S.A. and Ashiq, M., 2007. Semen production and productive life of Sahiwal bulls: relationship with genetic worth. *Pak. Vet. J.*, **27**: 20-24.
- Kondracki, S., Iwanina, M., Wysokinska, A. and Gorski, K., 2013. The use of sexual activity measurements to assess ejaculatory performance of boars. *Arch. Tierzucht.*, **56**: 1-13.
- Levis, D.G. and Reicks, D.L., 2005. Assessment of sexual behavior and effect of semen collection pen design and sexual stimulation of boars on behavior and sperm output - a review. *Theriogenology*, **63**: 630-642.
- Mahmood, S.A., Ijaz, A., Ahmad, N., Rehman, H., Zaneb, H., and Farooq, U., 2014. A study on relationships among age, body weight, orchidometry and semen quality parameters in adult Cholistani breeding bulls. *J. Anim. Pl. Sci.*, **24**: 380-384.
- Mandal, D.K., Nagpaul, P.K. and Gupta, A.K., 2003. Motion characteristics of murrh buffalo bull spermatozoa in various seasons and its relationship with functional integrity of the plasmalemma. *Theriogenology*, **60**: 349-358.
- Nordin, W., Hilimi, M. and Bongso, T.A., 1990. Semen characteristics related to age in growing Swamp buffalo (*Bubalus bubalis*). *Buffalo J.*, **6**: 161-166.
- Ott, R.S. and Memon, M.A., 1980. Breeding soundness examinations of rams and bucks. A review. *Theriogenology*, **13**: 155-160.
- Padrik, P., Hallap, T., Kaart, T., Bulitko, T. and Jaakma, U., 2012. Relationships between the results of hypo-osmotic swelling tests, sperm motility, and fertility in Estonian Holstein dairy bulls. *Czech. J. Anim. Sci.*, **10**: 490-497.
- Parkinson, T.J. and Vermunt, J.J., 2000. Bull management. In: *Proc. D.C. Vet. Assoc. N.Z.*, **17**: 203-219.
- Petherick, J.C., 2005. A review of some factors affecting the expression of libido in beef cattle, and individual bull and herd fertility. *Appl. Anim. Behav. Sci.*, **90**: 185-205.
- Pound, N., Javed, M.H., Ruberto, C., Anis, S.M. and Del Valle, A.P., 2002. Duration of sexual arousal predicts semen parameters for masturbatory ejaculates. *Physiol. Behav.*, **76**: 685-689.
- Qureshi, M.S., 2011. Preparation of semen extender, processing of semen and storage. In: (eds M.S. Qureshi), *Reproductive physiology of domestic animals*, Islamabad: HEC, pp. 127-129.
- Rehman, F.U., Qureshi, M.S. and Khan, R.U., 2014. Effect of soybean based extenders on sperm parameters of HolsteinFriesian bull during liquid storage at 4°C. *Pakistan J. Zool.*, **46**: 185-189.
- Rhen, T. and Crews, D., 2002. Variation in reproductive behaviour within a sex: neural systems and endocrine activation. *J. Neuroendocr.*, **14**: 517- 531.

- Sajjad, M., Ali, S., Ullah, N., Anwar, M., Akhter, S. and Andrabi, S.M.H., 2007. Blood serum testosterone level and its relationship with scrotal circumference and semen characteristics in nili-ravi buffalo bulls. *Pak. Vet. J.*, **27**: 63-66.
- Stranger, J.D., Long, V., Yovich, J.L. and Almahbobi, G., 2010. Hypo-osmotic swelling test identifies individual spermatozoa with minimal DNA fragmentation. *Rep. BioMed. Online*, **21**: 474-484.
- Younis, M., 1996. *Studies on semen quality, freezability and fertility of buffalo bulls during low and peak breeding seasons*. Ph.D. thesis, Univ. Agri. Faisalabad, Pakistan.
- Younis, M., Samad, H.A., Ahmad, N. and Ahmad, I., 2003. Effects of age and season on the body weight, scrotal circumference and libido in Nili-Ravi buffalo bulls maintained at the semen production unit, Qadirabad. *Pak. Vet. J.*, **23**: 59-65.