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Effects of Age, Breed and Scrotal Circumference Interactions on Sperm Morphology of Bulls Raised on Commercial Farms in Zambia

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ABSTRACT

The primary objective of this study was to investigate the effect of interaction of age, breed and scrotal circumference (SC) on the sperm morphological characteristics of bulls raised on commercial farms in Zambia. A total of 365 bulls comprising of 164 *Boran*, 139 *Bonsmara*, 35 *Tuli*, 12 *Santagetrudis*, 5 *Holstein Friesian* (HF) and 10 *Sussex* bulls were studied. Semen was collected once using an electro-ejaculator after which eosin-nigrosin stained semen smears were made. Thereafter, the eosin-nigrosin stained semen smears were examined under a microscope for abnormal spermatozoa. The interaction between age categories and the *Boran* bulls did not differ from the interaction of *Sussex* bulls older than 72 months (mo) of age in predicting sperm abnormalities ($P>0.05$). However, *Bonsmara and Sussex* bulls older than 72 mo of age had lower percentages of sperm abnormalities ($P<0.05$). Interaction of age and *Tuli* bulls showed high midpiece and tail defects (dag defects and simple coiled tails), total abnormal sperm, major defects and the Proximal cytoplasmic droplets (PCD), ($P<0.05$). *Santagetrudis* bulls between 48 and 60 mo of age showed an increase in the midpiece defects, total abnormal sperm, major defects and the PCD, ($P<0.05$), but lower than those of the interaction between age and the *Tuli* bulls. Higher coiled tails were found due to the interaction between age and HF bulls compared to age and the *Tuli* bulls ($P<0.05$). The interaction between age and SC had an effect on sperm abnormalities, ($P<0.05$). The study suggested that the interactions of age, breed and SC are important sources of variation in sperm morphology. However, further research is needed to determine the effect of minor nutritional differences on the sperm morphological characteristics on the farms.

Keywords: Age, breed, scrotal circumference, sperm abnormalities, interaction

Bull fertility is extremely important for optimal production of beef cattle bred by natural service under extensive management systems in tropical conditions (Nichi *et al.*, 2006). Natural service bulls should be selected carefully because their primary goal is to get cows pregnant as soon as possible after they become ready. For the bull to achieve this task, several factors must be met. These include; his semen quality, libido, mating ability and social ranking among bulls and cows. Therefore, beef bulls must pass a breeding soundness examination if they are to be included in the breeding programmes. Kondracki *et al.* (2014), stated that one of the elementary factors that determine the profitability of animal production is male and female fertility. Kastelic *et al.* (1996), reported that, a uniform intra-testicular temperature must be maintained between 2°C and 6°C lower than the body-core temperature for normal spermatogenesis in bulls. They further stated that factors such as the scrotum that holds the testes away from the body and the testicular vascular cone (TVC) that reduces testicular temperature by both counter-current heat exchange and radiation from the scrotal surface contribute to scrotal and testicular thermoregulation.

Sperm morphology and semen quality are some of the vital determinants of bull fertility or the cow conception rate. Therefore, assessment of sperm morphology is one of the most important aspects for semen evaluation and lower fertility in bulls and other species have been correlated to spermatozoa with high morphological abnormalities (Mandal, Kumar, & Tyagi, 2010). Menon *et al.* (2011), reported that sperm morphology is affected by factors such as breed, age, scrotal circumference (SC) and their interactions. Brito *et al.* (2002), reported that age and breed affected the characteristics of the scrotum, testes, sperm production and semen quality. Brito *et al.* (2002), further stated that the *Bos indicus* breeds are better adapted to the tropics and are more resistant to heat stress than the *Bos taurus* breeds because they usually have a smaller frame, greater skin surface to body size ratio, more sweat glands and lower thermogenesis. Results from a study by Vásquez *et al.* (2003), indicate that bulls need to have an average SC measurement for their age and breed if they are to produce normal semen.

Morphological abnormalities of sperm can be classified as; a) primary and secondary defects based on their presumptive origin, b) major and minor defects, a revised system where sperm defects are classified based on their perceived adverse effects upon male fertility and c) compensable and un-compensable semen traits according to a theoretical increase in numbers of functionally competent sperm that will or will not solve the problem. Compensable defects are those where the defective spermatozoa either do not reach the site of fertilization, or fails to initiate the fertilization process. Those that lead to failed fertilization or early pregnancy loss are termed as un-compensable (Alm-packalén, 2009). Brito *et al.* (2002) and Vilakazi, (2003) also reported abnormalities as minor and major. Some of the major abnormalities that were reported include; loose heads, double headed sperm, small abnormal heads, proximal cytoplasmic droplets (PCD), dag defects, pearshaped heads, knobbed heads, round heads, midpiece defects and contour on the heads. The minor defects that were reported include; microcephalic, macrocephalic, normal loose heads, distal cytoplasmic droplets (DCD) and tail defects. This study was conducted to determine the effect of the two-way interaction of age, breed and SC on sperm morphological characteristics of bulls raised on commercial farms in Zambia.

MATERIALS AND METHODS

Design and Sampling

The study was cross-sectional in nature. Proportional sampling was done from sixteen commercial farms in Zambia through an on-going bull testing exercise. Andrological examinations and semen sample collection were done between September and November, 2015. At least all bulls in the population were sampled since bulls are kept in low numbers. However, a 10% sampling fraction of the actual herd for the bull ratio was used where the bull population was high. Assuming low heterogeneity between herds a detection power (1-β) of 90% was used. The level of significance was set to 95% and the desired absolute precision at 5%. For the morphological analysis, a sensitivity of 80% and specificity of 100% for estimation purposes was assumed.

Breeds of Bulls Studied and feeding

A total of 365 bulls comprising of 164 *Boran*, 139 *Bonsmara*, 35 *Tuli*, 12 *Santagetrudis*, 5 *Holstein Friesian* (HF) and 10 *Sussex* breeds were studied. The bulls were kept under extensive conditions where free range grazing of mixed pastures was done and feed supplements were given to the animals (sunflower cake mixed with maize bran). Samples were collected immediately before the onset of the rains in the period between September and October 2015, which is the hot season.

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Inclusion and Exclusion criteria

Bulls included in the study were between 24 to 144 months of age with their eyes, legs, prepuce and penis, accessory sex glands (assessed through rectal examination) in good condition and free from diseases or lesions. Age and SC were each divided into four categories using the 25^{th}, 50^{th} and 75^{th} quartiles in SPSS version 20 as shown in table 1 below.

Table 1: Proportion of bulls according to Age and SC categories

	<36	Scrotal circumference (cm)				Total
		36 < to 38	38 < to 40	≥40		
Age	<48	29	29	11	5	74
	48 < to 60	33	28	15	12	88
	60 < to 72	13	14	15	12	54
	≥72	15	27	43	64	149
Total		**90**	**98**	**84**	**93**	**365**

Semen Collection

Semen samples were collected from the different bulls by the use of electro-ejaculator (EE) immediately after examination. The bulls were well restrained in a cattle crush pen using two pieces of strong wood behind the rear legs, this was to ensure safety of the operator during rectal palpation and to prevent back movement of the bull. The dung was removed from the rectum of the bull with a gloved hand, the accessory glands and pelvic urethra were then gently massaged for at least three minutes. Thereafter, a long electro-ejaculator probe was lubricated and fully inserted into the rectum. Then the operator performed slight and progressive increases in the electrical stimulation until there was penile protrusion, erection and finally ejaculation and collection of semen in a graduated collecting tube (Hafez & Hafez, 2000).

Slide preparation

The nigrosin-eosin stained smears were made in the field by firstly pre-warming the microscopic slide and nigrosin-eosin to body temperature to avoid cold shock. Then a drop of stain was pipetted onto the edge of the first slide and then a drop of semen next to the stain. The edge of second slide was placed into the drops of stain and semen and then rocked back and forth a few times to mix the sperm and stain. Subsequently, the second slide was used to make a smear across the surface of the first slide of the sperm and stain mixture, after which the slide was dried rapidly by waving it back and forth in the air and stored for Laboratory examination.

Laboratory examination of semen

The eosin-nigrosin semen stained smears were made in the field and taken to the laboratory for examination of sperm morphology. The examination of the field stained semen smears was done using a bright field microscope at x1000 with an oil immersion lens. At least 200 sperm were observed per slide in different fields and sperm morphological characteristics were observed and recorded

Statistical analysis

Multivariate Analysis of Variance (MANOVA) was conducted using the general linear model (GLM) procedure in SPSS version 20 to assess two-way interaction between the variables studied on sperm morphologic characteristics. Mean differences were detected using the Bonferroni test. A statistically significant MANOVA effect was observed, pillai's Trace $p<0.001$, the levene's test of equality of error variances was not significant ($p>0.05$) for all the defects studied signifying homoscedasticity among the groups of the dependent variables. The statistical significance was set to $P<0.05$ for all the assumptions.

RESULTS

Interaction between age and breed on sperm morphology

Table 2 shows the MANOVA results for the interaction between age and breed on sperm morphological characteristics. The results of the graphical representation of the interaction

Table 2: Interaction between breed and age on sperm abnormalities

Variables	Defect	Sum of Squares	Degrees of freedom	Mean square	F value	P value
Breed * Age	Total head	12.251	11	1.114	0.661	0.776
	Total midpiece	1902.046		172.913	4.489	0.000
	Total tail	1110.732		100.976	1.585	0.102
	Total normal	5407.207		491.564	2.524	0.005
	Total abnormal	5425.001		493.182	2.522	0.005
	Major defects	1979.834		179.985	3.104	0.001
	Minor defects	951.040		86.458	1.709	0.070
	Normal dead	421.991		38.363	0.636	0.798
	PCD	1899.567		172.688	5.441	0.000
	Dag	148.744		13.522	3.324	0.000
	Loose head	60.860		5.533	0.364	0.969
	Loose tail	5.503		0.500	1.095	0.365
	Coiled tail	151.117		13.738	2.929	0.001

between age and breed on total sperm abnormalities and major defects are shown in Figs. 1 and 2 below.

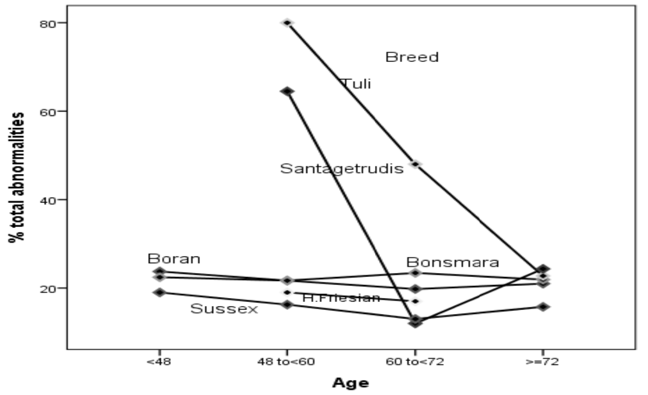

Fig. 1: Interaction between age and breed on % total abnormalities

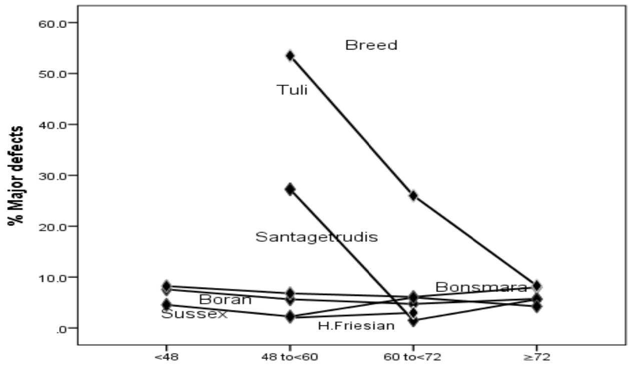

Fig. 2: Interaction between age and breed on major defects

Interaction between age and scrotal circumference on sperm morphology

Table 3 shows the MANOVA results for the interaction between age and SC on sperm morphological characteristics

The results of the graphical representation of the interaction between age and SC on total tail abnormalities and total abnormal sperm are shown in Figs. 3 and 4 below.

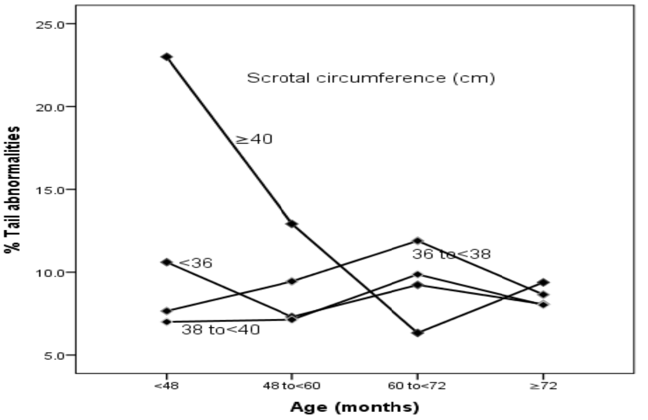

Fig. 3: Interaction between age and SC on total tail abnormalities

Table 3: Interaction between Scrotal circumference and age on sperm abnormalities

Variables	Defect	Sum of Squares	DF	Mean square	F value	P value
SC * Age	Total Head	13.561	9	1.507	0.894	0.531
	Total Midpiece	262.105		29.123	0.756	0.657
	Total Tail	1693.792		188.199	2.954	0.002
	Total Normal	5255.633		583.959	2.998	0.002
	Total Abnormal	5257.756		584.195	2.987	0.002
	Major defects	343.920		38.213	0.659	0.746
	Minor defects	1513.869		168.208	3.326	0.001
	Normal Dead	856.284		95.143	1.576	0.121
	PCD	188.104		20.900	0.658	0.746
	Dag	18.676		2.075	0.510	0.867
	Loose Head	63.533		7.059	0.465	0.898
	Loose tail	3.569		.397	0.868	0.554
	Coiled Tail	16.253		1.806	0.385	0.942

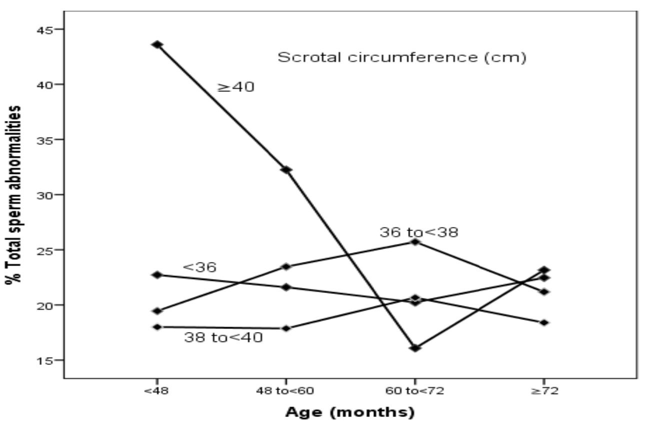

Fig. 4: Interaction between age and SC on total sperm abnormalities

DISCUSSION

The results of our study are in agreement with Vilakazi (2003), who found no significant interaction between age and breed on the percentage of minor defects but differ on the effect of interaction between breed and age on the percentage of abnormal sperm. In our study, bulls in the age category 48 to 72 months (mo) and *Tuli* breed had higher percentages of abnormal sperm while bulls of 72mo and *Sussex* breed had lower percentages of abnormal sperm. Therefore, it can be suggested that the *Tuli* breed of bulls have poor spermatogenesis with increasing age compared to the bull breeds that were studied. Menon *et al.* (2011), reported that, the cause of poor sperm morphology due to age could be as a result of testicular hypoplasia, testicular degeneration, and senescence of spermatozoa due to sexual inactivity as the bull grows older.

The findings of our study are in agreement with Coulter (1997) and Vilakazi (2003), who reported a significant interaction between age and breed on the percentage of major defects and semen quality. Vilakazi (2003), reported that young and older bulls had higher major sperm defects. Our study revealed that bulls aged 48 to 72mo and *Tuli* breed had the higher percentage of major defects. The age differences found between the two studies could be due to the variations in the breeds and the number of breeds studied coupled with differences in the age range of the bulls from the two studies. Sarder (2008), and Vilakazi (2003), stated that the major sperm defect should not exceed 20% if optimal fertility is to be achieved in bulls. The *Tuli* and *Santagetrudis* breeds of 48 to 72mo years had major defects above the critical value of 20%. However, the *Santagetrudis* breed had lower major sperm defects than the *Tuli* breed. The *Tuli* breed of 48 to<60mo of age had high midpiece defects which could have a genetic origin as suggested by Chenoweth, (2005).

In line with Madrid (1988), and Vilakazi (2003), major sperm defects such as loose heads, PCD, loose tail and midpiece defects are affected by age and breed interaction. The differences found on sperm abnormalities due to age and breed interaction could be due to differences in SC with age between breeds and the maturity type of the bull. Perumal (2014); Menon *et al.* (2011); Kabiraj *et al.* (2011); Vilakazi & Webb (2004); Vásquez *et al.* (2003) stated that SC is highly correlated with testicular parameters and body weight and that males with bigger testes produce more sperm than males with smaller testes depending on age and breed. Furthermore, Kealey *et al.* (2005), reported that, testicular development of bulls during the post-weaning period is affected by age and breed of the bull, environmental conditions and nutritional status and thus has a positive correlation with semen quality.

The *Tuli* bulls of 48 to 60mo old had high percentages of PCD. Menon *et al.* (2011), reported that the PCD are defects of spermatogenesis and signify failure in the epididymal maturation of sperm and a high percentage of this sperm defect results in impaired fertility. Menon *et al.* (2011), further reported that, when sperm with high PCD was used in *in-vitro* fertilisation (IVF), cleavage rates of embryos were poor. Banaszewska *et al.* (2015), reported that the frequency of the PCD and other morphological defects in the boars depended on the breed and age of the boar. Banaszewska *et al.* (2015), further stated that, cytoplasmic droplets contain numerous enzymes and receptors which in excessive quantities negatively affect male fertility and that the presence of the PCD in the middle piece can cause swelling and bending of the sperm tail and thereby preventing normal movement and hence reducing fertility. The findings of our study are in contrast to those of Sarder (2008), who found no significant effect of age on the PCD.

The findings of the interaction between age and SC on sperm morphology are in line with Coe (1999) and Kashoma *et al.* (2010), who recorded a variation in semen quality due to age and SC. Vilakazi (2003), reported that there is an increase in fat deposition in cattle with advancement of age and this fat may be deposited in the scrotum and hence cause poor thermoregulation leading to faulty spermatogenesis. Fat deposition in the scrotum affects testicular thermoregulation through decreased heat radiation from the scrotal neck (Brito *et al.* 2002a). Our study revealed that bulls less than 48mo with abnormally high SC values had high sperm abnormalities. The findings are in agreement with Vilakazi (2003), who found that bulls less than 48mo had high sperm abnormalities. Bulls less than 48mo had a higher SC for their age, this could reflect abnormal testicular tissue probably due fat deposit as a result of overfeeding of the bulls since the bulls where kept according to age groups at the various farms visited and supplemented differently. In line with the study by Sarder (2008), our study demonstrated that bulls of age 48 to<60mo with a SC of 38 to<40cm had the least tail defects such as the dag defects. Roberts *et al.* (2010), found high heritability estimates of the dag defects in the bulls that were studied and therefore producers could apply selection pressure against the trait in order to improve fertility. Chenoweth (2005), stated that the dag defect could be hereditary as it was observed in bulls that were full brothers. He further reported that, the dag defect has an environmental link with dietary zinc and may be present in normal sperm (<4%). However, levels above 50% can have serious implications on bull fertility.

CONCLUSION

The *Bonsmara* and *Sussex* bulls older than 72 months of age had the least sperm morphological

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abnormalities compared to the other bulls that were studied while the *Tuli* bulls of 48 to 72 months of age had higher sperm morphological abnormalities. Bulls older than 72 months with SC greater than 40cm had the least sperm morphological abnormalities. Therefore, breeding bulls must be selected carefully according to breed based on their minimum recommended SC for age.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

Alm-packalén, K. 2009. *Semen quality and fertility after artificial insemination in dairy cattle and pigs, Thesis*. University of Helsinki, Finland.

Banaszewska, D., Biesiada-Drzaga, B. and Andraszek, K. 2015. Frequency of Cytoplasmic Droplets Depends on the Breed and Age of Insemination Boars. *Folia Biologica (Krakow)*, **63**(1).

Brito, L.F.C., Silva, A.E.D., Rodrigues, L.H., Vieira, F.V., Deragon, L.A. and Kastelic, J.P. 2002. Effects of environmental factors, age and genotype on sperm production and semen quality in Bos indicus and Bos taurus AI bulls in Brazil. *Anim. Reprod.Sci.*, **70**(3–4): 181–190.

Brito, L.F., Silva, A.E., Rodrigues, L.H., Vieira, F.V., Deragon, L.A. and Kastelic., J.P. 2002a. Effect of age and genetic group on characteristics of the scrotum, testes and testicular vascular cones, and on sperm production and semen quality in AI bulls in Brazil. *Theriogenology*, **58**(6): 1175–1186.

Chenoweth, P.J. 2005. Genetic sperm defects. *Theriogenology*, **64**: 457–468.

Coe, P.H. 1999. Associations among age, scrotal circumference, and proportion of morphologically normal spermatozoa in young beef bulls during an initial breeding soundness examination. *J Am Vet Med Assoc*, **214**(11): 1664–1667.

Coulter, G.H. 1997. "Bull Fertility : BSE, Abnormalities, Etc." In *Proceedings, The Range Beef Cow Symposium XV December 9, 10 and 11, 1997, Rapid City, South Dakota Bull*.

Kabiraj, S.K., Hoque, S.A.M., Khandoker, M.A.M. and Husain, S.S. 2011. Testicular biometry and its relationship with body weight and semen output of black Bengal bucks in Bangladesh. *Journal of Cell and Animal Biology*, **5**(2): 27–32.

Kashoma, I.P.B. Luziga, C. and Mgongo, F.O.K. 2010. Bull selection and use for improved performance in pastoral herds of Tanzania. *Livest.Res.Rural. Dev.*, **22**(1).

Kastelic, J.P., Cook, R.B. and Coulter, G.H. 1996. Contribution of the scrotum and testes to scrotal and testicular thermoregulation in bulls and rams. *J.Reprod.Fertil,*. **108**(1): 81–85.

Kealey, C.G., Macneil, M.D., Tess, M.W., Geary, T.W. and Bellows, R.A. 2005. Genetic parameter estimates for scrotal circumference and semen characteristics of Line 1 Hereford bulls. *J.Anim. Sci*, **84**: 283–290.

Kondracki, S., Gorski, K., Wysokinska, A. and Jozwik, I. 2014. Correlation of ejaculate parameters and sperm morphology with the ejaculate volume of Pietrain boars. *Bulgarian Journal of Agricultural Science*, **20**(3): 703–709.

Madrid, N. 1988. Scrotal circumference seminal characteristics, testicular lesions of yearling Angus bulls. *AJVR*, **49**(4): 579–85.

Mandal, D.K., Kumar, M. and Tyagi, S. 2010. Effect of age on spermiogram of Holstein Friesian X Sahiwal crossbred bulls. *Animal*, **4**(4): 595–603.

Menon, A.G., Barkema, H.W., Wilde, R., Kastelic, J.P. and Thundathil, J.C. 2011. Associations between sperm abnormalities, breed, age and scrotal circumference in beef bulls. *Can. J. Vet. Res*, **75**: 241–247.

Nichi, M., Bols, P.E.J., Züge, R.M., Barnabe, V.H., Goovaerts, I.G.F., Barnabe, R.C. and Cortada, C.N.M. 2006. Seasonal variation in semen quality in Bos indicus and Bos taurus bulls raised under tropical conditions. *Theriogenology*, **66**(4): 822–828.

Perumal, P. 2014. Scrotal Circumference and Its Relationship with Testicular Growth,Age, and Body Weight in Tho Tho (*Bos indicus*) Bulls. *International Scholary Research Notices*, pp. 1–5.

Roberts, C.A., Geary, T.W., Macneil, M.D., Waterman, R.C., Roberts, A.J. and Alexander, L.J. 2010. Factors Affecting Spermatozoa Morphology in Beef Bulls. In *Proceedings, Western Section, American Society of Animal Science,.* **61**: 122–125.

Sarder, J.U. 2008. Effects of age , body weight , body condition and scrotal circumference on sperm abnormalities of bulls used for artificial insemination (AI) programme in Bangladesh. *Univ.J.Zool.Rajshahi Univ.*, **27**: 73–78.

Vásquez, L., Vera, O. and Arango, J. 2003. Testicular growth and semen quality in peripuberal Brahman bulls. *Livest.Res.Rural Dev.*, **15**(10).

Vilakazi, D.M. and Webb, E.C. 2004. Effect of age and season on sperm morphology of Friesland bulls at an artificial insemination centre in South Africa. *S.A.J.A.S.*, **34**(1): 62–69.

Vilakazi, M.M. 2003. *Factors affecting the quality of semen of A . I . dairy bulls in South Africa South Africa, Thesis.* University of Pretoria, South Africa.

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