Prevalence of spermatozoa morphologic defects from Zebu bulls under free mating system

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SUMMARY

Bulls are keys to fertility and genetic improvement of the total herd. Spermatozoa quality is among of major components for evaluation of bulls to be used as breeding bulls. This study was therefore carried out to determine the prevalence of spermatozoa defects in Tanzania shorthorn breeding bulls, and to determine if the defects vary with scrotal circumference (SC), age and testicular pathology. Age was determined by dentition and SC measured by using standard scrotal metal. Sperm cells were collected by epididymal aspiration procedure and stained with Carbol-fuchsin. One hundred spermatozoa were counted per slide and each classified into normal or abnormal. Three testicular samples were taken after slaughter and processed for histological examination by staining with hemotoxylin and eosin. Forty sections of seminiferous tubules per section were evaluated and classified into either normal or abnormal. After examinations, 169 and 134 bulls were classified as satisfactory (normal) and unsatisfactory (abnormal) breeders, respectively. The mean SC (in centimeters) and percentages spermatozoa defects in normal and abnormal bulls were 28.9±2.6 and 15.6±2.5 versus 22.0±3.6 and 39.0±9.1, respectively. Most frequently observed primary spermatozoa defects in normal bulls were Stump-tail, DAG defect and decapitated sperm head while the secondary spermatozoa defects were loose head, bent tail, and proximal and distal cytoplasmic droplets. Bulls with severe testicular lesions had high percentages of spermatozoa defects which increased with severity of testicular abnormalities (P < 0.01). The results confirm the absence of proper criteria for selecting breeding bulls and support the earlier evidence of association between SC, testicular pathology and spermatozoa defects in bulls.

Key words: Tanzania shorthorn zebu bull, spermatozoa defects, scrotal circumference, testicular pathology,

INTRODUCTION

Cattle production in Tanzania is generally based on Tanzania shorthorn zebu (TSHZ) extensively managed on natural pasture (MLDF, 2008). The breeding system is based on natural mating which accounts for approximately 85 - 95 of cows bred by using one or several bulls per herd (Shirima *et al.*, 2003; Mgongo and Kashoma, 2007). Under this condition together with lack of record keeping on farms make it difficult to assess the fertility of individual bulls based on the female's conception rate. Since a subfertile or infertile dominant bull can cause irreparable losses under extensive management conditions, the importance of conducting а Breeding Soundness Examination (BSE) cannot be overemphasized (Godfrey and Dodson, 2005; Sylla et al., 2007).

Breeding soundness examination of zebu bulls in the tropics is only carried out in the field (Chacón, 2001: Fitzpatrick et al., 2002). The objectives of BSE are to identify bulls that have acceptable reproductive efficiency and to find those that are subfertile or infertile and should be eliminated from the breeding stock (Chacón, 2001; Kennedy et al., 2002). Of the component of BSE, spermatozoa quality is of utmost importance in zebu bulls extensively reared in the tropics (Chacón, 2001). Evidence of a relationship between spermatozoa abnormalities (particularly those herd related to acrosome and proximal cytoplasmic droplet) and fertility (56 day non-return rate; NRR) of bulls has been reported (Söderquist et al., 1991). Furthermore, the percentages of morphologically normal or abnormal sperms are related to conception rate (Fitzpatrick et al., 2002). Consequently, sperm morphology seems to be the most reliable component of the spermiogramme, which together with an exhaustive clinical and genital evaluation of the animal provides detailed information for estimating the potential fertility of those bulls under field conditions in the tropics, disclosed as breeding soundness examination (Parkinson, 2004).

Various techniques have been used to collect semen from bulls, including artificial vagina, electro-ejaculation, transrectal massage of the ampullae, aspiration of sperm from caudal epididymis and testicular biopsy (Roberts, 1986; Goovaets et al., 2006). Nevertheless, due to management conditions (e.g. unavailabity of electro-ejaculator) and also to the hostile behavior of the breeding bulls (e.g handled captured under anaesthesia). or the collection of semen as part of BSE is done aspiration of caudal epididymis bv (Goovaets et al., 2006). Since, spermatozoa in semen samples obtained from the caudal epididymis have been reported to be as fertile as those in the ejaculate representing testicular as well as epididymal function (Borth and Oko, 1989). Furthermore, collection of semen from caudal epididymis offers the possibility to acquire and use genetic materials from elite males even after their death, and this semen can either be used fresh or be frozen and stored in genetic resource bank projects (Bartels *et al.*, 2001; Goovaets *et al.*, 2006).

Many studies have been carried out to describe the reproductive performance of female TSHZ cattle (Kanuya *et al.*, 2006a; Kanuya *et al.*, 2006b). However, no comparative studies have been carried out to describe the performance of TSHZ breeding bulls extensively reared under multiple breeding systems. The present investigation was therefore undertaken to study the frequencies of spermatozoa defects from TSHZ breeding bulls and to determine if these frequencies vary with scrotal circumference, age and pathologic conditions of testicles.

MATERIALS AND METHODS

Three hundred and three Tanzania shorthorn zebu bulls extensively reared under multiple male breeding systems were examined under field conditions. Eighty percent of bulls were brought for slaughter at Morogoro municipal abattoir from different parts of Tanzania and the remaining 20% were from herds around Morogoro region. The examination included clinical and genital examination, semen collection and evaluation. and tissue testicular collection and histopathological evaluation.

Clinical and genital examination

The author performed the detailed clinical healthy and genital examinations of the bulls at the farms and the abattoir (during ante-mortem examination for bulls brought for slaughter). The examination included clinical health, body condition (visual assessment) and age (by dentition through visual assessment of eruption and wear of using a standard dentition method described by Goetz, (1979)). The clinical genital examination involved measurement of scrotal circumference (using a standard metal-tape) and testicular scrotal consistency (determined subjectively by palpation) and classified as "normal', "soft" and "hard"

Semen collection and evaluation

Following the clinical genital examination, sperm cells (epididymal semen) samples were obtained using a modified epididymal aspiration procedure as described by Khorram et al (2001). In detail, a site on the bottom aspect of a testicle nearest to the epididymis was shaved caudal and disinfected with methylated spirit. A sterile 18-gauge needle was then threaded half to one centimeter deep into the caudal of the epididymis. In each case a sterile 5 ml syringe was used to aspirate semen by suction. The aspirated semen was placed on a clean microscopic glass slide, diluted approximately equal amount of with normal saline, thoroughly mixed and finally spread over the slide before air drying. The air dried slides were fixed with 0.2% glutaraldehyde solution. Staining was according to Boguth, (1951), in brief, the slides were covered with carbol fuschin solution for five minutes, washed with 1% acid-alcohol (1.0 ml glacial acetic acid and 99.0 ml ethyl alcohol 90-95%) solution for five minutes, placed in methylene blue solution for two minutes and finally washed with absolute alcohol before air drving. The smears were ultimately examined for spermatozoa abnormalities at 1000X magnification using a Minitube® HT007 dark phase microscope (Minitube Abfull – und Labortechnik GmbH, Tieferbach. Germany). At least 100 spermatozoa were counted in each sample, and classified as either: normal spermatozoa, spermatozoa with abnormal heads or tails, or spermatozoa with proximal or distal cytoplasmic droplets (values expressed as percentages).

Testicular tissue collection and histopathologic evaluation

Immediately after slaughter at the abattoir, the scrotum (containing the testes and epididymis) was excised, wrapped in a plastic bag and transported in a cool box to the laboratory. In the laboratory, the scrotum was excised and testes together with epididymis were exposed. Three testicular tissue samples were taken from dorsal, middle and ventral regions and fixed in Bouin's solution. After 48 hours of fixation, the samples were washed with running water for 6 hours. The tissues were then trimmed and preserved in 70% ethyl temperature alcohol at room until processed. The standard procedure for processing fixed tissue for histological examination was used and the tissues were stained with haematoxylin and eosin. Forty sections of seminiferous tubules per each tissue were examined for pathological changes by using a light microscope at X1000 magnification. The changes were loss of germinal epithelium, loss of spermatocytes and/ or spermatogonia, vacuolation, presence of pyknotic nuclei, and presence of giant cells. only sustentacular cells in seminiferous tubules. The percentages of germinal epithelial loss (PGEL) described by Veeramachian et al. (1986) were used to grade tissues either normal (less than 25% testicular tissue loss) or abnormal (more than 25%

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testicular tissue loss). The abnormal testicular tissues were further graded into five grades, namely, 1 = moderate degeneration, 2 = moderate hypoplasia, 3 = severe degeneration, 4 = severe hypoplasia, and 5 = mixture (at different rates) of degeneration and hypoplasia.

Furthermore, after thorough examinations, bulls were categorized into two groups namely, normal bulls (healthy, good body condition, free of any clinical congenital or inflammatory diseases in their reproductive organs, histologically normal testicles and low percentage of spermatozoa defects) and abnormal bulls (healthy, good body condition, free of any clinical congenital or inflammatory diseases in their reproductive organs, histologically abnormal testicles and high percentage of spermatozoa defects). In summary, 55.8% of bulls qualified to be satisfactory breeders (normal bulls) and 44.2% unsatisfactory breeders (abnormal bulls).

Statistical analysis

Data was handled by Microsoft excel where, statistical analyses were performed by using Statistical Analysis System (SAS, 1999) windows program. Assumptions of normality and equal variances between age groups for each spermatozoa defect were investigated using DISCRIM procedure of SAS. Number of bulls having each spermatozoa defect and their corresponding testicular histopathologic features were calculated for each age group. Each spermatozoa defect was ranked by overall prevalence from least to most prevalence, using a RANK procedure of SAS. The relationship between spermatozoa defects and testicular histopathologic features at different age categories were determined procedure CORRE using of SAS. Differences of spermatozoa defect between age groups and testicular pathology were tested using 2-way analysis of variance, with age and PGEL as main effects.



Figure 1. Spermatozoa smears. Spermatozoa with bent tail (A) and stump tail (B). (Carbolfuchsin stain; x1000

RESULTS

Detailed data on number of bulls and percentages of spermatozoa defects are summarized in Table 1. Table 2 summarizes the effects of testicular conditions on the percentages of spermatozoa defects. Table 3 illustrates the percentages of specific spermatozoa defects in relation to age and soundness of

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bulls observed in this study. Typical examples of spermatozoa abnormalities as classified in this study are depicted in Figure 1.

Relationship between spermatozoa defects and age

The mean percentages of spermatozoa defects in normal bulls were 15.6±2.5 (mean and standard deviation). Bulls below 2 years old had the highest number of total spermatozoa defects while bulls with 4 and above years had the lowest. Among the primary spermatozoa defects, Stamp-tail, decapitated sperm head, DAG defects and pear shaped each accounted for less than 2% of total abnormalities and did not significantly contribute to total spermatozoa abnormalities of any age Regarding group. to secondary spermatozoa abnormalities, bulls with normal testicles had few abnormalities in all age groups and the mostly observed secondary spermatozoa abnormalities in all age groups were loose head and bent tails.

In regards to abnormal, young bulls (less than 2 years old) had the highest scores of defects while older bulls (4 and above vears) had the lowest. The primary spermatozoa defects were observed in high percentages (2.2 ± 1.0) versus (1.5 ± 0.6) of their age mates in normal group. The commonly observed primary spermatozoa abnormalities were Stump tail, DAG defect, pear - shaped heads and decapitated sperm heads. Among the secondary spermatozoa defects, loose heads and bent tail were commonly observed in all age proximal groups while and distal cytoplasmic droplets was higher in younger bulls (below 2.0 years old), moderate in 2.0 to 2.5 years old bulls and low in other age categories.

Age categories	in Number of	Percentages of ±SD*)	spermatozoa	abnormal	lities (Mean
years	bulls	Primary	Secondary	Tertiary	Total
Below 2.0					
Normal bulls	11	1.7 ± 0.8	14.1 ± 2.7	2.9 ± 2.0	16.0 ± 2.8
Abnormal bulls	14	2.1 ± 0.8	32.8 ± 8.6	3.4 ± 1.7	39.2 ± 9.7
2.0 - 2.5					
Normal bulls	26	1.5 ± 0.6	13.6 ± 1.6	2.0 ± 1.0	16.4 ± 2.1
Abnormal bulls	13	2.2 ± 0.9	34.1 ± 10.7	3.9 ± 1.6	39.9 ± 10.8
2.5 - 3.0					
Normal bulls	36	1.6 ± 0.5	13.8 ± 2.0	1.9 ± 1.1	15.8 ± 1.9
Abnormal bulls	24	2.1 ± 0.9	33.5 ± 9.0	3.1 ± 1.6	38.6 ± 9.0
3.0 - 4.0					
Normal bulls	57	1.6 ± 0.6	13.0 ± 1.8	1.9 ± 0.9	15.7 ± 2.0
Abnormal bulls	28	2.4 ± 1.2	33.0 ± 7.4	4.5 ± 2.2	39.8 ± 8.8
Above 4.0					
Normal bulls	33	1.5 ± 0.5	12.6 ± 1.6	1.6 ± 0.7	14.5 ± 1.7
Abnormal bulls	14	2.0 ± 1.3	30.2 ± 6.5	3.7 ± 2.5	35.7 ± 6.8
Total					
Normal bulls	163	1.5 ± 0.6	13.4 ± 2.7	2.1 ± 1.0	15.6 ± 2.5
Abnormal bulls	94	2.2 ± 1.0	33.0 ± 8.4	3.8 ± 2.1	39.0 ± 9.1

Table 1. Percentage of spermatozoa abnormalities in Tanzania shorthorn zebu bulls

* SD = Standard Deviation

Relationship between spermatozoa defects and scrotal circumference

The mean scrotal circumference in bulls with normal testicles was 28.9 ± 2.6 centimeters (mean and standard deviation). The scrotal circumference increased with age and positively correlated with age (r=0.69; p<0.01; n=169). However, the increment in scrotal circumference was greater in young bulls (less than 3 years) than in older bulls (above 3 years). In regards to bulls with abnormal testicles, the mean scrotal circumference was 22.0±3.6 centimeters (mean and standard deviation). Similarly, the scrotal circumference in this group positively correlated with age (r=0.63; p<0.01; n=134). Bulls with large scrotal circumference (normal testicles) had lower mean percentages of spermatozoa defects (15.6±2.5) compared age mates with small scrotal circumference (abnormal testicles) which had higher (39.0±9.1) percentages of spermatozoa defects.

Testicular condition	Number of bulls	Percentage of s (mean ± SD*)	of spermatozoa defects	
		Primary	Secondary	
Normal bulls	169	1.5 ± 0.6	13.4 ± 2.7	
Diseased bulls				
Moderate degeneration	26	2.1 ± 1.8	34.8 ± 8.7	
Moderate hypoplasia	24	2.2 ± 1.4	43.6 ± 7.3	
Moderate mixture of	36	2.3 ± 1.3	38.7 ± 5.1	
degeneration and hypoplasia				
Severe hypoplasia	14	Azoospermia	Azoospermia	
Severe degeneration	12	Azoospermia	Azoospermia	

* SD = Standard Deviation



Figure 2. Histological section of seminiferous tubules with a severe degeneration in a 37 - 48 months old zebu bull (A) and with a moderate degeneration (multiple vacuolation) in above 48 months zebu bull (H&E, x400)

Relationship between spermatozoa defects and testicular pathology

Pathologic changes representing six grades, namely, moderate degeneration, moderate hypoplasia, severe degeneration, severe hypoplasia and mixture of degeneration ad hypoplasia at different rates were observed. Bulls with high percentages of germinal epithelial loss (severe degeneration or hypoplasia) had no spermatozoa (azoospermia). Table 2 summarizes the mean percentages of spermatozoa defects according to testicular conditions. In general, bulls with moderate degeneration, moderate hypoplasia or a moderate mixture of degeneration and hypoplasia had higher percentages of spermatozoa defects as compared with normal testicles. Typical examples of testicular lesions observed in this study, are depicted in Figures 2 and 3. When the testicular histopathologic grades spermatozoa were correlated to Pearson's abnormalities. negative а correlation was revealed (r=-0.16; P<0.19; n=303).



Figure 3: Histological section of seminiferous tubules with a severe hypoplasia in a 25 - 30 months old zebu bull (A) and with a moderate mixture of hypoplasia and degeneration in above 48 months old zebu bull (H&E, x400)

Specific spermatozoa defects

The most prevalent primary spermatozoa defects observed in bulls with normal testicles included Stump-tail (47.3%; 80 out of 169 bulls), DAG defect (13.6%; 23 out of 169) and decapitated sperm head (13.6%; 23 out of 169 bulls). For secondary spermatozoa defects, loose head (separated normal head) were more prevalent than bent tail (distal coiled tail) in all age categories. The prevalence of proximal and distal cytoplasic droplets was high in young bulls (less than 2.0 years), moderate in middle aged bulls (between 2.0 and 4.0 years) and lower in older (4.0

and above years) bulls. In bulls with abnormal testicles, Stump tail was the common type (57.5%; 54 out of 94 bulls) of primary defect observed in all age categories while DAG defect, pear – shaped heads and decapitated sperm heads were least observed in all age categories. Table 3 summarizes the mean percentages of specific spermatozoa defects according to age and soundness of bull.

DISCUSSION

In the present study, majority of bulls (88.8%; n=269) were more than two years old and only few (11.2%; n=34) were

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below two years old. Most (80%) bulls examined were bought from farmers and submitted to the abattoir by businessmen. Farmers submitted on voluntary basis animals for sale on the understanding that bulls were healthy and could fetch high amount of money. In Tanzania, bulls used as herd males are never examined for breeding soundness during their lifetime. Therefore both fertile and infertile or subfertile bulls are equally used as breeding stock as it was observed by Mgongo and Kashoma, (2007).

Table 3. Prevalence of spermatozoa abnormalities in Tanzania shorthorn zebu bulls

Spermatozoa	Age category (years)					
abnormality	< 2.0	2.0 – 2.5	2.5 – 3.0	3.0 – 4.0	>4.0	
Decapitated sperm						
head						
Normal bulls	1.0 ± 0.3 (3)	1.0 ± 0.0 (4)	1.0 ± 0.0 (6)	1.0 ± 0.2 (4)	1.0± 0.0 (6)	
Abnormal bulls	1.0 ± 0.3 (4)	1.0 ± 0.3 (4)	1.0 ± 0.0 (7)	1.0 ± 0.3 (4)	1.0 ± 0.0 (6)	
DAG defect						
Normal bulls	1.0 ± 0.0 (2)	1.0 ± 0.4 (6)	1.0 ± 0.2 (5)	1.0 ± 0.0 (5)	1.0 ± 0.4 (5)	
Abnormal bulls	1.0 ± 0.0 (3)	1.0 ± 0.4 (4)	1.0 ± 0.2 (6)	1.0 ± 0.0 (6)	1.0 ± 0.4 (4)	
Stump tail defect						
Normal bulls	2.0 ± 1.2 (6)	2.1 ± 0.6 (10)	1.9 ± 1.1 (15)	1.0 ± 1.0 (33)	2.2 ± 1.2 (16)	
Abnormal bulls	2.0 ± 1.2 (6)	2.1 ± 0.6 (9)	1.9 ± 1.1 (15)	2.0 ± 1.0 (16)	2.2 ± 1.2 (8)	
Bent tail (distal coiled						
tail)						
Normal bulls	4.0 ± 2.9 (11)	4.2 ± 3.4 (26)	4.1 ± 2.9 (36)	2.0 ±3.2 (57)	4.4 ± 3.2 (33)	
Abnormal bulls	13.0 ± 2.9 (14)	13.2 ± 3.4 (13)	13.1 ± 2.9 (24)	13.0 ±3.2 (28)	13.4 ± 3.2 (14)	
Loose head (separated						
normal head)						
Normal bulls	5.6 ± 2.3 (11)	5.5 ± 2.6 (26)	5.6 ± 2.1 (36)	5.3 ± 2.3 (57)	5.2 ± 2.2 (33)	
Abnormal bulls	18.6 ± 2.3 (14)	13.2 ± 3.4 (13)	18.6 ± 2.1 (24)	18.3 ± 2.3 (28)	18.2 ± 2.2 (14)	
Proximal cytoplasmic						
droplet						
Normal bulls	1.0 ± 1.0 (5)	1.3 ± 0.6 (13)	1.0 ± 0.3 (16)	1.0 ±0.1 (21)	1.0 ± 0.0 (12)	
Abnormal bulls	2.0 ± 1.0 (8)	1.3 ± 0.6 (7)	1.0 ± 0.3 (9)	1.0 ± 0.1 (17)	1.0 ± 0.0 (10)	
Distal cytoplasmic						
droplet						
Normal bulls	2.0 ± 0.0 (4)	1.5 ± 1.0 (12)	1.0 ± 0.0 (19)	1.0 ±0.0 (32)	1.0 ± 0.0 (16)	
Abnormal bulls	$2.0 \pm 0.0(7)$	1.5 ± 1.0 (9)	1.0 ± 0.0 (12	1.0 ± 0.0 (22)	1.0 ± 0.0 (11)	

Data are mean \pm SD. Number in parentheses is number of bulls

morphologic defects Reports of in spermatozoa for large numbers of bulls of various ages and different testicular conditions are limited. Johnson et al. (1998) reported that 68% of spermatozoa were normal in 10 to 12 months old bulls considered to have average to high fertility, 73.3% in 13 to 18 months old bulls, 77.6% in 19 to 24 months old bulls and 76% in above 25 months old bulls. By comparison, in bulls with normal testes in our study,

84% of spermatozoa were normal in below 2 years old bulls, 83.6% in 2.0 to 2.5 years old bulls, 74.2% in 2.5 to 3.0 years old bulls, 74.3% in 3.0 to 4.0 years old bulls and 75.5% in above 4 years old bulls. The higher percentage of normal spermatozoa for bulls in our study is likely attributable to normalcy testicles as revealed by testicular histopathologic features.

The result of this study indicated that the percentage of normal spermatozoa increased with the age of the bull. Bulls at or below two years old have a higher percentage of abnormal spermatozoa probably due to pubertal changes. The higher percentages of abnormal spermatozoa reported in this study do not agree with other studies (Makarechian and Farid, 1985; Makarechian et al., 1985), which reported that 18 months old normal bull have reached puberty, and further increases in the percentage of morphologic defects in spermatozoa are not expected. This is most likely attributable to differences between breeds in reaching puberty, Tanzania shorthorn zebu bulls reach puberty at late age as compared with other zebus, and the high percentage of spermatozoa abnormalities at 2 years is probably due to pubertal changes.

The primary spermatozoa defects such as decapitated sperm head, DAG defects and pear shaped each accounted for less than 2% of total abnormalities and did not significantly contribute to total spermatozoa abnormalities of any age category. DAG defect development in the epididymis is believed to be due to exposure to abnormal epididymal secretion (Barth and Oko, 1989) and is commonly associated with disturbances to scrotal/testicular thermoregulation in bulls (Kalistelic et al., 1997). On the other hand, Stump-tail abnormality accounted for more than 2% in more than 49% of bulls examined. Stump-tail defects are indicative of abnormal spermatogenesis and are rarely found in large numbers affecting relatively small numbers of bulls (Stolla et al., 1996). This was not true in our study, as indicated by high prevalence and high proportion of bulls having the defect in their spermatozoa. However, further studies should be conducted to establish if the Stump-tail defect is an inherited defect in

Tanzania shorthorn zebu and to estimate its effects on bull's fertility.

A small (4 to 6%) percentages of loose/detached head and bent tail defects were found in the semen of clinically normal bulls in this study, and these defects are sometimes (in low percentages) caused preparation during of slides (Harasymowycz et al., 1976). However, abnormal bulls in this study had higher percentages of loose head (>18%) and bent tail (>13%). The higher percentages of loose head and bent tail are associated with infertility and these are caused by testicular degeneration, hypoplasia or low concentration of circulating testosterone due to variety of adverse stimuli, such as fever, scrotal insulation or stress in bulls (Barth and Oko, 1989; Barth and Bowman, 1994; Parkinson, 2004).).

Cytoplasmic droplets (proximal and distal cytoplasmic droplets) are often found in higher numbers in spermatozoa from young bulls, because their testicles are immature (Arteaga et al., 2001; Chacón, 2001). In our study, both proximal and distal cytoplasmic droplets were found most commonly in bulls less than 2 years old. but the prevalence decreased significantly in mature and old bulls. The decrease in number of spermatozoa affected with cytoplasmic droplet defects and the proportion of bulls affected with the defects as bulls age increased are probably the result of testicular maturation.

Conclusion

In conclusion, the mean spermatozoa defects values of normal TSHZ bulls from this study are similar to that observed in other zebu bulls reared under extensively management with multiple - male breeding systems. The results also confirm that good numbers of bulls (about 44%) that are used as breeding bulls are infertile or subfertile.

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Therefore, proper identification and elimination of these bulls is important for increased reproductive performance in pastoral herds. However, further studies should be conducted to establish the causes and effects on bull's fertility of the most prevalent primary spermatozoa abnormality, the Stump – tail defect observed in this study.

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