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SPERM MORPHOLOGY AND SPERM QUALITY OF BULLS RAISED ON COMMERCIAL FARMS IN ZAMBIA

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ABSTRACT
Sperm morphology and sperm quality was studied from 365 bulls raised on commercial farms in Zambia. Bulls were classified as sound or unsound for breeding based on general physical examination coupled with the morphological evaluation of the eosin nigrosin stained smears. Single semen samples were collected from each bull by electro-ejaculation and mass motility estimated with a 0-5 scale immediately after collection and then stained with the eosin-nigrosin stain. Sperm morphology was determined using a light microscope at X1000 under oil immersion. A total of 200 sperm were counted and abnormalities expressed as a percentage of the total number of counted spermatozoa. The most common defects were the simple bent tails (4.42 ±0.31%), normal dead (7.51 ±0.40%), proximal cytoplasmic droplets (PCD) (3.50 ±0.32%), loose dead heads (1.65 ±0.20%) and the distal cytoplasmic droplets (DCD) (1.36 ±0.16%). Defects where then classified into tail, midpiece and head defects. A final classification into major and minor defects was done. Tail defects were the most predominant with (9.00 ±0.43%), followed by the mid-piece defects (4.50 ±0.35%) and head abnormalities (0.78 ±0.07%) respectively. Based on sperm morphology, 71 bulls failed to pass the examination representing 19.5% of the entire bull population studied while 294 bulls representing 80.5% passed the examination. Breeding bulls with a BCS of 2.0 had the highest percentage of dead sperm (11.20 ±1.40%) and the highest percent of major defects (10.17 ±1.43%) while those of BCS 3.5 had the lowest percent dead sperm (5.11 ±0.66%) and major defects (4.27 ±0.78%), ($p<0.05$). The highest percent PCD (5.35 ±1.13%) and lowest percent PCD (2.26 ±0.44%) was found in bulls of BCS 2.0 and 3.5 respectively ($p<0.05$). The *Sussex* breed had the highest head abnormalities (2.05 ±1.05%) and highest major defects (14.60 ±4.90%) ($P<0.05$) while the *Santagetrudis* breed had the lowest head abnormalities of (0.50 ±0.18%) ($p<0.05$) compared with other breeds. Bulls with a BCS of 2.0 were more likely to have a watery semen sample relative to a creamy sample compared to bulls with a BCS of 3.5 ($p<0.05$). Bulls of BCS 3.5 had the highest semen volume while those of BCS 2.0 had the lowest ($p<0.05$). Semen colour was affected by BCS ($p<0.05$). In conclusion, BCS and breed are important predictors of sperm morphology while BCS is an important predictor of sperm quality.

KEY WORDS: Breed, sperm quality, body condition score, scrotal circumference, age.

INTRODUCTION

Breeding soundness refers to a bull's ability to get cows pregnant (Berry *et al*., 2011). Semen is the secretion of male accessory reproductive glands and contains the male gametes (spermatozoa) produced in the testis (Parkinson, 2004). Breeding Soundness of a bull implies a complex interaction of factors such as physical and reproductive health, libido and mating ability, semen quality and interactions among animals in the breeding herd (Chacon *et al*., 1999). Major influences on herd fertility and production are exerted by the bull, whether this bull is bred with many females using assisted reproductive technologies such as artificial insemination or with relatively few females via natural service. Relatively little selection pressure for reproductive traits has been placed on most bull populations and the use of several bulls as practiced routinely by commercial producers makes it difficult to identify sub-fertile bulls and hence the increasing demand for breeding soundness evaluation (BSE) (Younquist and Threlfall, 2007).

It is important that a bull be free from any disease for it to be fertile. Furthermore, three more attributes are needed for the bull to be fertile, and these are; good libido, physical soundness and good semen quality. These three attributes must be held in the fore-front of all decisions regarding herd sire selection and BSE. Management factors, body condition, environmental stress and photoperiod related endocrine changes may result in differences in the proportion of bulls with satisfactory breeding soundness classification at different times of the year (Barth and Waldner, 2002). Hossein-zadeh and Akbarian, (2015), reported that, change in body condition score over time reflects both body composition and energy balance, which in turn are critical for metabolic stability, health and fertility. Strous, (2010), stated that about 20% of bulls may have reduced fertility in a population. However, in a study conducted by Barth and Waldner, (2002), 19.2% of bulls

were classified as unsatisfactory potential breeders and 14.9% as questionable breeders.

It is important that the bull is examined thoroughly, ensuring that the bull is able to eat, see, smell and move around. This is because any factor that reduces the efficiency of these activities will have a negative effect on the bulls breeding ability (Younquist and Threlfall, 2007). Other factors to consider are body condition, age and breed because these have an effect on the quality of semen that the bull will produce at ejaculation (Ahmed *et al*., 2014). It is vital that a thorough history of the bulls' recent illness is obtained. This is because semen quality and/or quantity may be reduced as a result of prior illness (Noakes *et al*., 2003). Bittar *et al*. (2015), demonstrated that the infection of bulls with *Trypanosoma vivax* reduced libido in bulls and subsequent reduction in semen quality. He found that there was some degeneration, diffuse or inter lobar inflammatory infiltration in the bovine testicles.

There are a number of classification systems for morphological abnormalities of sperm, these are; i) primary and secondary defects, which classify sperm abnormalities on the basis of their presumptive origin, ii) major and minor defects, a revised system where sperm defects are classified in terms of their perceived adverse effects upon male fertility and iii) 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) also reported abnormalities as minor and major and some of the abnormalities that he found were, tailless sperm, also called disintegrated or decapitated sperm which are early indicators of testicular degeneration as a result of heat stress and under nutrition, abnormal acrosomes such as knobbed acrosomes, ruffled acrosomes, bent and coiled tails which could be the most common abnormality found in the ejaculate of bulls and may be associated with reduced fertility and cytoplasmic droplets which are an indication of incomplete maturation of sperm within the epididymis. Other abnormalities as reported by Vilakazi, (2003) include; abnormal loose heads, double forms, degenerative heads, distal droplets, loose acrosomes, normal loose heads, pyriform (pear-shaped heads) and dag defects.

METHODOLOGY

Study design and sampling

The study design was cross sectional in nature. The study was performed in selected commercial farms in Kabwe, located in the Central Province of Zambia with geographical coordinates 14° 26' 0" South, 28° 27' 0" East and Choma lying south west of Lusaka, on the Lusaka-Livingstone road in Southern Province. The town lies on the south east edge of the Kafue flats wetland (15° 52′ S 27° 46′ E). Various commercial farms were visited and andrological examinations and semen collection were done on each bull between September and November 2015. Proportional sampling was conducted according to target population given that Choma has more commercial farms than Kabwe and hence more farms were selected from Choma than Kabwe. Since bulls are kept in low numbers, we sampled at least all bulls in the population. However, were the bull population was high, we used a 10 percent sampling fraction of the actual herd for the bull ratio. Assuming low heterogeneity between herds, we used a detection power (1-β) of 90 percent and the level of significance (α) was at 95 percent and the desired absolute precision at 5 percent. For the morphological analysis, we assumed a sensitivity of 80 percent and specificity to be at 100 percent for estimation purposes.

Inclusion and Exclusion criteria

The bulls included in the study were between the ages of 2 to 12 years and had their eyes clear of lesions, good rear leg conformation, general health was good, the prepuce and penis were free from disorders such as hematomas, lacerations and growths because these may prevent successful service. The accessory sex glands were free from abscesses and inflammation, the epididymis did not have any palpable masses and the bulls were not cryptorchids.

Breeding soundness evaluation (BSE)

The BSE was applied to sires intended for natural mating under extensive pasture management conditions with or without supplementation. Assessment of the management of the bulls was done based on the feeding system (supplemented or not). The breeding history and thorough clinical inspection was done. Before doing any andrological evaluation of the bulls, description of each bull was done and recorded: breed, identification number/mark and age, body condition score and scrotal circumference were recorded. Body condition was scored from 1 to 5 according to the system devised by Nicholson and Butterworth, (1986), where a score of less than 3 was considered poor and above 3 as normal with a BCS of 5 as obese. General clinical inspection and specific examination of the reproductive organs was performed and results recorded. The date of examination, name of farmer/ owner and location was also recorded. The general clinical inspection included the eyes and the musculoskeletal system. The specific examination included the inspection of the prepuce and penis (was only done if penile protrusion occurred during semen collection). The scrotum and its contents were examined and the SC measured using a tape placed at the widest mid-scrotal point.

Semen collection

Semen samples were collected from the different bulls once by the use of an electro-ejaculator 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.

Evaluation of fresh semen under field conditions

Macroscopic analysis: volume and colour of semen were observed visually, with diagnostic value to a certain extent, for functioning of accessory glands (Dhurvey *et al*., 2012). The volume of ejaculate was measured directly with the help of graduated collection vial and its colour was observed and recorded.

Microscopic analysis: A drop of semen was put on the slide for observation. Mass activity was subjectively estimated in a small magnification (X100) in the light microscope using a 0-5 score in non-cover slipped samples (Chenoweth, 2004). The inference of the 0-5 scoring system is as shown in the table below.

TABLE 1: Grading of semen according to mass motility *(*Hossain et al., 2012)

scale	grade	inference
5	+++++ (Excellent)	Extremely rapid swirls and eddies, vigorous movements, sperms are extremely rapid and changing constantly (more than 80% vigorous motion)
4	++++ (Very good)	Well defined, strong waves with rounded turns (70-80% vigorous motion)
3	+++ (Good)	Slow waves visible (50-70% motion)
2	++ (Fair)	Many motile but no wave motion (30-50% motion)
1	+ (Poor)	Few motile sperm (<30% in weak motion)
0	0 (Zero)	No motility observed

The eosin-nigrosin 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 the 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 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

Morphology: The eosin-nigrosin stain was used to assess the sperm morphology because it is effective, simple and in addition to allowing sperm to be readily visualised, it is so called "live –dead" stain allowing one to assess membrane integrity at the same time as morphology since it distinguishes viable from non-viable spermatozoa. Examination of the field stained smears was done using a bright field microscope at X1000 with an oil immersion lens. At least 200 spermatozoa per sample were observed in different fields and classified for normality and abnormality (Chenoweth, 2004).

STATISTICAL ANALYSES

Mean percentages (±SEM) were calculated for every sperm abnormality and volume. Means were summarised separating the bulls into categories, based upon the following variables: Breed, Age <48 months (mo), 48 to <60 mo, 60 to <72 mo and ≥72 mo, SC <36cm, 36 to<38 cm, 38 to<40cm, ≥40 cm and BCS of 2.0, 2.5, 3.0 and 3.5. Association between the categorised variables was analysed using the Fishers exact test and/or the Chi square test. An analysis of variance (ANOVA) was performed between categories using the Bonferroni test for the comparison of means. Bulls with <70% normal sperm morphology were classified as unsound and those with ≥70% as sound (Parkinson, 2004; Chenoweth, 2002 and Kennedy *et al*., 2002). The effects of variables studied on semen colour categories were assessed using multinomial logistic regression, Using SPSS version 20.0. Age and SC were each merged into two categories while breed type was not included in the multinomial regression model because many cells had zero frequencies and breed type could not be merged in order to fill the empty cells.

RESULTS

Defects were categorised into, Head (acephalic sperm, knobbed head, pearshaped, macrocephalic, microcephalic, double headed, round head, contour on head and narrow head), midpiece (thickened midpiece, coiled midpiece, dag and the swollen Midpiece) and tail defects (loose dead/normal heads, simple bent tails, double tailed, coiled end piece and straight tail) and the proximal and distal cytoplasmic droplets (PCD and DCD). Final classification into major and minor defects was done according to Chenoweth, (2002). Major defects included the loose dead heads, acephalic sperm, knobbed head, pearshaped, double headed, contour on head, PCD, dag defects, thickened midpiece, coiled midpiece, round head and swollen midpiece while minor defects included the macrocephalic, microcephalic, narrow head, loose normal heads, simple bent tails, terminally coiled tails, DCD, double tailed and straight tails. The means of the defects according to categories as stated above are shown in tables 2 and 3.

The mean percentages (±SEM) of sperm abnormalities according to BCS are shown in table 2 below.

TABLE 2. Mean percentages (±SEM) of sperm abnormalities according to BCS

Variable		Dead	Head	Midpiece	Tail	Major	Minor	PCD	DCD
BCS	2.0	11.20±1.40^{a}	0.93±0.17^{a}	6.01±1.15^{a}	10.87±1.18^{a}	10.17±1.43^{a}	7.59±0.80^{a}	5.35±1.13^{ac}	1.35±0.33^{a}
	2.5	7.22±0.46^{b}	0.92±0.13^{a}	4.30±0.57^{a}	9.09±0.73^{a}	6.60±0.69^{b}	7.63±0.69^{a}	3.54±0.54^{a}	0.99±0.21^{ab}
	3.0	7.18±0.80^{b}	0.57±0.10^{a}	4.42±0.55^{a}	8.06±0.69^{a}	6.05±0.62^{b}	6.93±0.61^{a}	3.14±0.48^{a}	2.04±0.39^{c}
	3.5	5.11±0.66^{b}	0.73±0.16^{a}	3.55±0.74^{a}	8.74±1.07^{a}	4.27±0.78^{b}	8.68±1.09^{a}	2.26±0.44^{b}	0.82±0.22^{a}

$^{a\text{-}c}$ Means in the same category and column with a distinct letter (s) are statistically different (P<0.05)

The bulls with a BCS of 2.0 had the highest percent of Dead sperm and the lowest was seen in bulls with a BCS of 3.5 ($p<0.05$). The highest percent major defects and PCD were recorded in bulls with a BCS of 2.0 and the lowest in those with 3.5 ($p<0.05$). Highest DCD were observed in bulls with a BCS of 3.0 and the lowest in the 3.5 category ($p<0.05$). Although not significant ($p>0.05$), the highest mean head, midpiece and tail defects were found in bulls with a BCS of 2.0. No significant mean differences were found on sperm abnormalities due to SC ($p>0.05$). Regardless of the insignificant finding, the highest mean percent of dead sperm, tail defects, PCD and minor sperm defects were found in bulls with a SC greater or equal to 40 cm while the highest head defects in those bulls with a SC less than 36 cm.

Mean percentages (±SEM) of sperm abnormalities according to breed are presented in table 3.

TABLE 3. Mean percentages (±SEM) of sperm abnormalities according to breed

Breed	Dead	Head	Midpiece	Tail	Major	Minor	PCD	DCD
Boran	7.70±0.58[a]	0.77±0.09[a]	3.73±0.43[a]	9.31±0.66[a]	5.92±0.56[a]	7.84±0.65[a]	3.27±0.42[a]	1.52±0.26[a]
Bonsmara	7.78±0.64[a]	0.67±0.09[a]	5.22±0.61[a]	8.52±0.71[a]	7.43±0.72[a]	6.90±0.55[a]	3.58±0.52[a]	0.84±0.17[a]
Sussex	6.85±1.73[a]	2.05±1.05[b]	9.45±4.67[a]	12.70±2.69[a]	14.60±4.9[b]	9.57±2.07[a]	8.30±4.72[a]	1.60±1.07[a]
Santagetrudis	10.7±1.65[a]	0.50±0.18[a]	7.25±2.45[a]	11.54±2.88[a]	8.88±2.75[b]	10.04±2.73[a]	5.17±2.07[a]	2.13±0.99[a]
Friesian	5.50±1.85[a]	1.30±0.97[ab]	2.10±1.29[a]	9.00±3.05[a]	2.60±1.17[a]	9.80±2.93[a]	2.10±1.29[a]	1.60±1.26[a]
Tuli	5.17±1.81[a]	0.93±0.24[ab]	3.30±0.90[a]	7.94±0.90[a]	4.43±0.93[a]	7.63±0.92[a]	2.53±0.78[a]	2.31±0.79[a]

[a-b] Means in the same category and column with a distinct letter (s) are statistically different ($P<0.05$)

The *Sussex* breed had the highest head and major defects than the other breeds while the *Santagetrudis* breed had the lowest head abnormalities ($p<0.05$). No mean differences were found on dead, midpiece, tail, minor defects, PCD and DCD due to breed ($p>0.05$).

No mean differences were found on all the sperm abnormalities in the different age categories studied ($p>0.05$). Table 4 shows the mean percentages (±SEM) of semen volume according to BCS.

TABLE 4: Mean percentages (±SEM) of semen volume according to BCS

BCS	Mean ±SEM	Minimum	Maximum
2.0	4.58±0.28[a]	0.5	10.0
2.5	5.28±0.21[ab]	1.0	15.0
3.0	5.44±0.26[ab]	0.5	15.0
3.5	6.02±0.42[b]	1.0	20.0

Means in the same category and column with a distinct letter (s) are statistically different ($P<0.05$).

Bulls of BCS 3.5 had the highest mean volume while those of BCS 2.0 had the lowest ($p<0.05$). No differences were found in the mean semen volume due to breeds of bulls, age and SC categories ($p>0.05$).

The Proportion of semen colours for semen produced during the study are shown in Table 5.

TABLE 5: Proportion of semen colours for semen produced during the study

Parameter	Bull breed	Attributes	Count	Proportion (%)
Semen colours	*Boran* (n=164)	Creamy	63	38.4
		Milky	70	42.7
		Thin milky	8	4.9
		Watery	23	14.0
	Bonsmara (n=139)	Creamy	51	36.7
		Milky	50	36.0
		Thin milky	6	4.3
		Watery	32	23.0
	Sussex (n=10)	Creamy	6	60
		Milky	2	20
		Thin milky	1	10
		Watery	1	10
	Santagetrudis (n=12)	Creamy	3	25
		Milky	5	41.7
		Thin milky	0	0.0
		Watery	4	33.3
	Holstein Friesian (n=5)	Creamy	3	60
		Milky	1	20
		Thin milky	0	0.0
		Watery	1	20
	Tuli (n=35)	Creamy	9	25.7
		Milky	18	51.4
		Thin milky	4	11.4
		Watery	4	11.4
	Overall (n=365)	Creamy	135	37.0
		Milky	146	40.0
		Thin milky	19	5.2
		Watery	65	17.8

n =number of bulls

No effect was found due SC and age on milky and thin-milky semen colour relative to creamy semen colour ($p>0.05$). However, bulls with a BCS of 2.0 were more likely to have watery semen colour relative to a creamy colour compared to bulls with a BCS of 3.5 ($p<0.05$), (OR,3.31; 95 % CI, 1.12 to 9.78). In general, 37.0% of the breeding bulls produced semen with creamy colour, 40.0% with milky colour, 5.2% with thin-milky colour and 17.8% with watery semen colour. No association was found between breed and semen colour ($p>0.05$, $p=0.407$).

DISCUSSION

A total of 71 bulls out of 365 were classified as having morphologically abnormal sperm representing 19.5% of the entire bull population studied. The PCD was found to be the most predominant defect of the midpiece defects followed by the dag defect. A higher prevalence of PCD in bulls with a BCS of 2.0 could be due to delayed maturation of the spermatozoa due to endocrine alteration as a result of poor or low nutrition in these bulls *Chacon et al.* (1999). An interesting finding was that bulls with a SC of greater or equal to 40cm were found to have more PCD as opposed to the findings of Chacon, (2001), who reported that bulls with a SC of less than 30cm as having more PCD. This signifies that bulls with an abnormally large SC had more immature sperm. Parkinson, (2004) reported that PCD are regarded as more serious than the DCD and are a defect of spermatogenesis especially when they occur in large numbers with other mixed abnormalities, and poor fertility has been observed in ejaculates with as little as 5-10%.

The most common defects were the simple bent tails, similar finding where the simple bent tail was the most common defect found in the bulls raised in the tropics was reported by Chacon, (2001) and Menon *et al.* (2011), in beef bulls regardless of breed and age. Menon *et al.* (2011), reported that the bent tail defect causes sub-fertility in natural service bulls. Parkinson, (2004), reported that the bent tail can arise due to abnormal seminal pH especially in animals with seminal vesiculitis or could be due to testicular degeneration in the company of mixed abnormalities. This defect could also be a tertiary defect post-ejaculation which arises when sperm are subjected to osmotic or temperature shock.

A higher percentage of loose dead/normal head was recorded in the *Sussex* breed compared to the other breeds. It is not uncommon to find smaller percentages of loose heads in the bull ejaculate. However, the common causes for large number of spermatozoa with loose heads in a semen sample are testicular hypoplasia, testicular degeneration and senescence of spermatozoa due to sexual inactivity (Menon *et al*., 2011). The *Sussex* breed had the highest head and major defects than the other breeds while the *Santagetrudis* breed had the lowest head abnormalities. Enciso *et al*. (2011), found a clear relationship between major sperm defects and poor deoxyribonucleic acid (DNA) quality. Furthermore, he suggested that the presence of a disrupted sperm DNA molecule ultimately leads to the production of lethal or sub-lethal sperm cell and thus the aetiology of this DNA fragmentation needs further studies.

A higher percentage of loose dead heads (tailless) sperm and major defects were found in bulls with a BCS of 2.0. The highest DCD were observed in bulls with a BCS of 3.0 and the lowest in the 3.5 category. This is a signal that a bull must have a good BCS if the semen is to be of good quality with fewer abnormalities. Chacon *et al.* (1999) reported that, a low BCS could signify low nutritional levels which may delay sexual maturity and cause testicular degeneration in bulls as a consequence of endocrine alterations. The findings of this study are in contrast with those of Chacon *et al.* (1999), who found no correlation between BCS and sperm morphology. Body condition score was a significant predictor of sperm quality and morphology and it was seen clearly that bulls with a lower BCS had a higher probability of failing the BSE examination compared to bulls with a good BCS. Therefore BCS must be within the recommended and accepted limits according to breed and age for a bull to have higher chances of producing semen of higher quality. These findings are in line with previous reports such as that of Barth and Waldner, (2002) where having a poor BCS reduced the chances of a bulls being classified satisfactory for breeding. Therefore, it can be suggested that a good BCS should be taken into consideration when selecting bulls for both AI and natural mating programmes.

No significant difference was seen due to age and SC on all the sperm abnormalities, this is in agreement with the findings by Menon *et al.* (2011), who found no significant effect of age and SC on all the sperm abnormalities in the beef bulls studied but is in contrast with the findings of Sarder, (2008), who reported significant effects of age and SC on the sperm abnormalities of the breeding bulls and bulls from which AI semen was obtained.

In our study semen colour was classified as creamy, milky, thin milky and watery. The findings of the present study are in agreement with previous reports as that of Lemma & Shemsu, (2015), who found that 31.2% of the breeding bulls had creamy semen colour and that those with milky colour were the most predominant and is also in line with the findings by Alemu *et al*. (2014) and also similar to the findings by Gebre Medhin *et al.* (2007), who found that 41.2% of the bulls had milky semen colour and 37.3% with creamy colour. A huge variation between these studies was seen in the percentage of the watery semen colour found, in our study having the highest percentage of 17.9% compared to 3.92% by Gebre Medhin *et al*. (2007) but closer to 12.8% by Alemu *et al*. (2014). The other colour proportions differ with other studies with our study having non for yellowish and bloody semen colour while Gebre Medhin *et al.* (2007), found 7.84% bloody and 9.80% yellowish semen colours in the *Jersey*, *Holstein Friesian* (HF) and *HF x local* breeds studied while Lemma & Shemsu, (2015), found 8.7% yellowish and 0.02% bloody. In contrast with the study by Lemma & Shemsu, (2015), no association was found between breed and semen colour in our study. Ayana, (2004), found that semen with the spermatozoa concentration of one million to 1.2 million per cubic millimetre or higher was found in the semen samples classified as having a creamy consistency while the

spermatozoa with concentration of 500,000 to 600,000 per cubic millimetre as thin milky and those with the spermatozoa concentration of less than 300,000 spermatozoa per cubic millimetre as watery, translucent or clear. It can thus be suggested that bull semen which is concentrated has creamy or milky colour consistency and therefore the importance of determining the bull semen colour cannot be overemphasized. The presence of semen with abnormal colours indicates problems associated with the process of spermatogenesis and pathologies on the accessories and reproductive organs and the reproductive passage ways (Gebre Medhin *et al*., 2007). The variations in the present study findings with the previous reports might be due to the differences in the breeds studied, the subjectivity of the colour determination method and environmental factors.

The volume of semen did not differ significantly among the six breed types, this is in line with the results described by Gebre Medhin *et al*. (2007); Boujenane & Boussaq, (2014) and Hossain *et al*. (2012) who reported no significant differences among the breeds studied but is in contrast with the studies by Alemu *et al*. (2014);Lemma and Shemsu, (2015) and Kunowska-Slósarz *et al*. (2015), who found significant volume differences among the bull breeds studied. This insignificant finding could be due to individual differences in the performance, as well as differences in the sample sizes and the number of breed types studied. Gebre Medhin *et al*. (2007), stated that differences in semen volume could be due to differences in the nutritional status of the animals, geographical locations, month of semen collection, collection method, bull handling during collection procedure and the frequency of ejaculation.

Body condition score was an important predictor of semen volume in the study where bulls with a BCS of 3.5 had a higher semen volume compared to bulls with a BCS of 2.0. The findings of our study are in line with that of Beran *et al*. (2011), who found a significant association between BCS and quantitative traits of bull semen. The low BCS was attributed to poor nutrition in our study because bulls with any disorder or disease where not included in the study.

Semen volume has been previously reported to increase with increasing age by Brito *et al*. (2004). In our study, no influence of age was found on semen volume, this is in agreement with the findings of Ruttle *et al.* (1975) who found no significant age effect on the different age groups of the bulls maintained under range conditions in the tropics. Similar findings were reported by Parthenis, (1951) who found no influence upon the volume of the beef bulls due to age.

In contrast to the studies by Ha *et al*. (2012) and *Latif et al.* (2010), no association was found in our study between semen volume and SC. Alexander, (2008), reported that bulls not meeting the minimum SC according to breed and age should fail the BSE examination because SC is positively correlated with bull fertility.

CONCLUSION

Body condition score and breed were the significant predictors of sperm abnormalities. Only BCS had an effect on semen volume and semen colour.

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