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Semen quality of progeny-tested breeding bulls maintained at the National Artificial Insemination Centre, Arusha, Tanzania

[1,2*]NGODA P, [3]KASHOMA I, [1,4]MSALYA G

[1]Department of Animal, Aquaculture, and Range Sciences (DAARS), Sokoine University of Agriculture (SUA), P.O Box 3004, Chuo Kikuu, Morogoro, Tanzania;
[2]National Artificial Insemination Centre, P.O Box 7141 Arusha, Tanzania;
[3]Department of Reproduction, College of Veterinary Medicine and Biomedical Sciences, SUA, P.O Box 3020, Chuo Kikuu, Morogoro, Tanzania.
[4]Tanzania Dairy Board (TDB), P.O Box 17029, Dodoma, Tanzania

[*]**Corresponding author**: peterpatricia78@yahoo.com

Abstract

The success or failure of artificial insemination (AI) depends on the quality of semen used. This study was conducted to investigate the effect of breed, age, collection period, and ejaculate number on semen quality collected from 15 breeding bulls available at the centre with different ages (4 Friesian, 4 Ayrshire, 3 Jersey, 2 Simmental and 2 Boran breeds) maintained at National Artificial Insemination Center (NAIC), Arusha, Tanzania. A total of 600 ejaculates were collected for four weeks per month consecutively from January to May 2022, weekly per bull and twice a day; morning (6.00 - 9.00 AM) and evening (3.00 - 6.00 PM) sessions). The ejaculates were first examined visually for volume, color, foreign bodies, and consistency whereas sperm concentration (SPC), progressive motility (PM) and total motility (TM) were assessed using Computer Assisted Semen Analysis System (CASA). Viability/vitality (SPVI) and morphology (SPNR) of sperms were analyzed microscopically after staining with Eosin-Nigrosin. Data were subjected to SAS program and results showed that ejaculate volume, progressive motility, total motility, sperm viability, and morphology were significantly ($P<0.05$) affected by age and collection period. Simmental, Friesian, and Ayrshire bulls produced more voluminous semen than Jersey and Boran bulls. Jersey bulls had higher ($P<0.05$) sperm concentrations and live spermatozoa as compared to other bulls. Boran and Jersey's bulls exhibited higher PM and TM than the other bulls. The period of semen collection (morning versus evening) negatively affected semen volume, total motility, and semen viability in which morning harvests performed better than evening collections. Middle aged bulls (3-5 years) produced semen of higher quality compared to other groups (< 3 years and > 5 years old bulls). In conclusion, age, breed, period of semen production and ejaculate number revealed significant effect on bovine semen characteristics.

Keywords: *Age; breeding bulls; collection period; ejaculate volume; semen quality*

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Introduction

In Tanzania, the contribution of the livestock sector to the gross domestic product (GDP) is estimated at 7 percent (NBS, 2021). It is an important sector contributing to nutrition, and the country's economy with a growth rate of 5 percent per year (WBT, 2021). In particular, the livestock sector plays a significant role as the source of livelihood among the underserved communities in rural areas of Tanzania (MLF, 2021). Cattle are the major and most important species of livestock estimated to be 33.9 million in number, making Tanzania ranked second in terms of their population in Africa after Ethiopia (URT, 2021). However, the economic potential of the cattle industry and

the livestock sector as a whole have not been fully exploited due to the low productivity of the animals, in particular, cattle have slow growth, poor reproductive rates, low off-take rates and high mortality rates (Smith *et al*., 2018). In addition to that, breeding, reproduction and fertility are also among the major challenges in the cattle industry leading to animals with low productivity and possible occurrences of inbreeding (Lawan *et al*., 2020).

Despite the Tanzanian government's efforts on promoting the use of artificial insemination (AI) practices for the improvement of animal productivity, the adoption rates have been slow depending on farming communities, social-economic factors like low-income low level of education, and lack of awareness on AI (Temba, 2011; Chi and Yamada, 2002). The Tanzania Livestock Sector Analysis (LSA) and Livestock Master Plan (TLMP) suggest that the sector will contribute to about 19 percent of the gross domestic product (GDP) if various interventions will be put in place to enhance cattle breeding and realize its genetic gain, robust and practical germplasm delivery technologies including (AI) and bull selection (Michael, 2018). The use of AI in the dairy cattle industry is of substantial economic benefits including genetic improvement for milk production, control of venereal diseases and inbreeding, facilitating management of cattle herd fertility, increased efficiency of bull usage, and availing geographical restrictions (Vishwanath, 2003; Bearden *et al*., 2004; Lemma and Shemsu, 2015; Lamb and Mercadante, 2006). However, the success of AI as a breeding tool depends on the quality of semen used (Christensen *et al*., 2011; Ahmed *et al*., 2016).

Semen quality is assessed based on fresh and frozen semen characteristics through macroscopic or microscopic examination. Currently, different laboratory methods such as Computer-assisted sperm analysis (CASA) (Amann and Waberski, 2014), Flow Cytometry Analysis (FCA) (Han, 2014), Luminometry (Gibbs and Kennebunk, 2001), and Enzyme-linked Immunosorbent Assay (ELISA) (Konstantinou, 2017) are commonly employed. These methods provide better estimates of the fertilizing ability of individual spermatozoa before insemination (Rodriguez-Martinez, 2003). Among these, CASA is considered to be the most efficient method for assessing the quality of fresh semen and can measure multiple dimensions of sperm fertility with high precision and accuracy (Tanga *et al*., 2021). On the other hand, semen quality is affected by many factors including breed or the genetic constitution of the animal (breed type), reproductive age and health status, management, technical skills, season and time of collection or period (Koivisto *et al*., 2009; Mandal *et al*., 2010; Mukhopadhyay *et al*., 2010; Ahmad *et al*., 2011). These factors influence the best value of the semen collected. Reproductive age determines the effective period when the bull can maximize production proficiently for producing frozen semen (Mandal *et al*., 2010; Ahmad *et al*., 2011). Suyadi *et al*., (2022) reported that age has a significant effect on the volume of fresh semen produced. Therefore, in the selection of bulls for semen collection age must be one of the criteria for better quality and quantity for frozen semen production. Furthermore, Fuerst-Waltl *et al*., (2006) observed that genetics, temperature, season, collecting, and feeding frequency are additional factors that can also affect the quality of semen and its fertility during application. Further information concerning the influence of period and age on semen characteristics also occurs in different breeds and species (Mandal *et al*., 2010; Ahmad *et al*., 2011). Hence, Artificial Insemination centers as producers of frozen semen are compulsorily required to produce and supply semen with good fertility. Thus, factors affecting semen production and quality such as breed, age and collection period are important as may affect semen quality (Karoui *et al*., 2011; Felton-Taylor *et al*., 2020; Seyoum *et al*., 2021).

For many years farmers and stakeholders along the AI service chain were complaining (unpublished) about poor conception rates when using semen produced from our National Artificial Insemination Center. This made them trust more on semen imported from other countries resulting in poor adoption and little trust in semen from NAIC for Artificial Insemination (Issuja, 2012). Hence, following these complaints it was important to undertake the study to find out the truth whether the challenge with semen from NAIC may be having poor quality which leads to poor conception rate or it's because of other issues along the production and distribution chain. This is part of a large and ongoing study aimed at understanding the AI supply chain in the country and what would be the cause of

poor adoption. For this specific study investigation was based on the animals (age, breed, CP and ejaculate number). Results obtained will be evidence for the subject matter with regard to quality of NAIC semen. Hence, create way forward for researchers and academicians, also awareness and trust among the farmers, artificial inseminators and stakeholders on the quality of semen from NAIC and establish useful information for the center, researchers and academicians on the appropriate age, breed and collection time for harvesting semen which are of high quality which is the major determinant of fertility and conception rate.

Materials and methods

Description of the Study Area

This study was conducted at the National Artificial Insemination Centre (NAIC), Arusha, Tanzania. NAIC is a government institution founded in 1974 responsible for the supervision and management of AI services in the country by raising the animals specifically bulls from which semen is collected, collection, processing, storage, selling, and transportation of semen to various areas serving both local and international semen markets. The centre is located at USA River, an area about 20 kilometres east of Arusha City, north-eastern Tanzania between latitudes 3.5^{o} and 3.7^{o} south of the Equator and longitudes 36.5^{o} to 37.5^{o} east of Greenwich. The area receives biannual (short and long) rainfall with cold and warm seasons. The cold season starts from June to August while the warm season falls from September to May.

Bull selection and Management

During the onset of the experiment, fifteen mature breeding bulls of three different age categories of below three years, 3 – 5 years and above five years managed at NAIC, Arusha, Tanzania were selected and used for this study. These bulls represented five breeds used as semen donors namely Holstein Friesian, Ayrshire, Jersey, Simmental and Boran. At the onset and throughout the study, all the breeder bulls were in good health status, maintained in individual pens and under identical management conditions. The animals received uniform management including feeding with hay and green forages and supplemented with concentrates fortified with minerals and vitamins. Water was supplied *ad libitum* to all bulls via water points and was allowed to exercise by walking in their paddocks every day. Preventive measures against internal and external parasitic infestation as well as vaccination against common diseases were also undertaken regularly.

Semen collection

Semen ejaculates were collected twice a day; morning session (6.00 to 9.00 AM) and evening session (3.00 to 6.00 PM) a weekly interval for 5 consecutive months from January to May 2022. On the day of semen collection, bulls were first allowed to go through preparatory measures including shaving around the prepuce, showering, drying, and cleaning. The bulls were then led to making at least two false mounting before ejaculation, at third time the bulls were directed on teaser bull and the semen was collected with the help of a well sterilized pre-warmed (42 to 45°C) artificial vagina (AV) fitted with a graduated and transparent (for easy observation of content) semen collection tube. The whole process of semen collection was done by well-trained personnel to avoid misleading of SOPs and incidence of contamination. Immediately after collection, the initial parameters of each semen ejaculate were macroscopically evaluated by laboratory technician for volume, (if its above 2mls), color, (if it has acceptable milk, creamy, light creamy and light-yellow color) if it appears different from named color means the semen do not qualify, and consistency by looking whether there are any foreign materials like hair, red particles, dust and pulse. Finally, the qualified ejaculates were kept in a water bath at 37°Ca few minutes (not more than 10 minutes) for further analysis.

Figure 1

Micrograph of bulls' spermatozoa, red colour indicates rapid progressive, green colour for medium progressive, blue colour for low progressive and yellow colour for immotile (400x)

Assessment of sperm motility and concentration

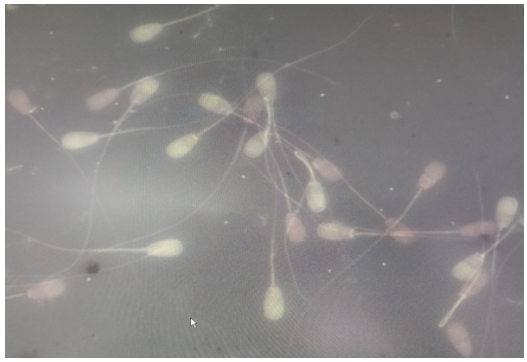
Spermatozoa kinetic parameters and concentration were evaluated microscopically by Sperm Class Analyzer CASA system (SCA) which consists of a phase-contrast microscope Olympus BX 51 microscope (Olympus, Japan), connected to the Sperm Vision™ (Minitüb, Tiefenbach, Germany) and a computer for saving and analyzing the data. Prior to CASA analysis the microscope stage and the slide were pre-warmed at 37°C. Briefly, an aliquot (10 µL) of semen was pipetted and placed on a warmed microscope slide at 37°C and covered with a coverslip (18×18 mm). The sperm kinetic parameters analyzed were total motility (%, proportion of moving cells against non-moving cells) and progressive motility (%, percentage of cells moving progressively). For each sample kept for analysis with green filter, 5 to 8 fields were captured under 400x magnifications. Sperm images were digitized for analysis of the kinematic patterns using the Sperm Vision™ software. The mean values were calculated for each of the assessed parameters; total motility (TM%), progressive motility (PM%) and concentration (M/ml).

Analysis of Sperm Viability/Vitality

The vitality of spermatozoa was assessed by the Eosin-Nigrosin staining technique based on the degree of membrane permeability of dead sperms. Briefly, one drop of each semen sample was mixed with an equal volume of Eosin-Nigrosin stain and gently swirled to mix and 5µL of each stained sample was smeared onto a clean microscopic slide. Each slide was air-dried followed by an examination of at least 200 spermatozoa per slide at a magnification of 1000x magnification under oil immersion with a bright field microscope. Sperms stained red or pink were classified as dead while sperms with light whitish coloration were classified as live. The percentage vitality was calculated by counting the number of membrane-intact spermatozoa divided by the total number of spermatozoa multiplied by 100 percent.

Figure 2

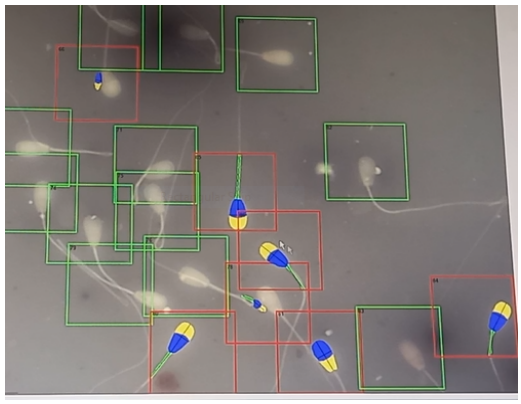
Photograph of bull's sperm viability /vitality, red/pink colour (Eosin stained) considered as dead spermatozoa and whitish coloration (without Eosin penetration) considered as live spermatozoa (Eosin Nigrosin stains, 1000x)



Assessment of Sperm Morphology

The spermatozoa morphological characteristics were also assessed using the Eosin-Nigrosin at 1000x magnification with under oil immersion with blue filter after staining. Based on the morphological characteristics, the spermatozoa were classified into seven categories such as normal (intact in characteristics), abnormal head (acrosome defects/nuclear pouches), detached head, proximal cytoplasmic droplets, bent midpiece, bent tail and coiled tail. Normal morphology was calculated by counting the number of normal spermatozoa divided by the total number of spermatozoa captured multiplied by 100 percent.

Figure 3. Photograph of bull's sperm morphological defects, without colouration represents normal, with colour represents abnormal (Eosin Nigrosin stains, 1000x immersion oil)



Statistical analysis

Semen production records were typed into a spreadsheet (MS-excel) and were subjected to One Way Analysis of Variance (ANOVA). The results were expressed as the mean and standard error of the mean using a statistical analysis system (SAS, 2004) to determine whether there were significant differences between the levels of the class variables at P value <0.05. In the statistical model, the effect of non-genetic factors on the semen quality parameters including ejaculate volume (VOL), sperm concentration (SPC), progressive motility (PM), total motility (TM), semen viability/vitality (SPVI), and sperm morphology (SPNR). The following statistical model was used.

$Yijkl = \mu + Bi + Aj + Ck + En + eijkn$

Where;

Yijkl - observed semen parameters
μ - overall mean;
Bi-fixed effect of the i^{th} breed class
Aj - fixed effect of the j^{th} age
Ck- fixed effect of the k^{th} production period
En- fixed effect of n^{th} ejaculate number and
eijkn - a random residual effect.

The interaction between different factors was tested and found not too significant and was therefore, removed/not used in the model.

Results

In this study, 600 ejaculates were collected from 15 bulls for the duration of five consecutive months (January to May 2022). The results showed that volume, total motility and sperm viability/vitality were significantly different *(p<0.05)* among the five cattle breeds (Table 1). Significant differences *(P<0.05)* in volume among breeds were observed. The mean semen volume in this study was variable ranging between 4017.9mm^3 in Boran breed bulls to 5369.6mm^3 in Simmental bulls. Simmental bulls had significantly *(P<0.05)* higher volume compared to Jersey and Boran, while slightly similar semen volume was obtained in Friesian (4453.4mm^3) and Ayrshires (5002.3mm^3) bulls. Jersey bulls had higher *(P=0.05)* sperm concentration (1293.72) than that of other bull breeds. Higher progressive motility was observed in semen collected from Boran (65.6%) bulls and the lowest was obtained in Friesian (60.7%). In contrast, semen collected from Jersey (87.80%) bulls exhibited significantly higher *(P<0.05)* total motility than that of Friesian and Ayrshires (81.23 and 82.09 respectively). Whereas, Simmental, Friesian, Ayrshire and Boran produced semen with equal total motility. A percentage live spermatozoon was higher in Jersey bulls followed by Friesian with the lowest in Simmental bulls. Regarding the sperm normalcy, Boran was at top rank in producing semen with higher percentage (93.70%) normal spermatozoa compared to other bull breeds. However, all bull breeds exhibited morphological defects not exceeding 20%.

With regard to morphological characteristics of semen, our results are presented in Table 2 and we clearly show that the semen collected at NAIC from several bulls were normal morphologically by over 83 percent. Various abnormal characteristics were also detected and as shown in Table 2.

Table 1

Comparison of fresh semen quality traits recorded in five bull breeds kept at National Artificial Insemination Centre

	Bull breeds				
Parameter	Friesian	Ayrshire	Jersey	Simmental	Boran
VOL (mm^3)	4453.38±398.19^{ab}	5002.27±360.5^{ab}	4191.38±398.19^{b}	5369.6±470.8^{a}	4017.88±456.27^{b}
SPC (M/ml)	1202±115.84^{a}	1198.7±134.1^{a}	1293.72±148.1^{a}	1102.23±175.1^{a}	1202.68±169.7^{a}
PM (%)	60.65±1.98^{a}	62.85±2.29^{a}	63.77±2.53^{a}	61.05±2.99^{a}	65.62±2.89^{a}
TM (%)	81.23±1.86^{b}	82.09±2.15^{b}	87.80±2.37^{a}	81.54±2.81^{ab}	81.24±2.72^{ab}
SPVI (%live)	88.24±1.64^{ab}	86.1±1.89^{b}	91.57±2.09^{a}	85.18±2.48^{b}	85.83±2.4^{ab}
SPNR (% normal)	86.56±1.27^{ab}	87.68±1.47^{a}	83.96±1.63^{ab}	90.20±1.92^{ab}	93.70±1.86^{ab}

^{ab} values across rows with different super scripts are significantly different *(P < 0.05).* Values in the table represent the mean ± SEM. VOL= volume; SPC = sperm concentration; PM = progressive motility; TM = total motility; SPVI = sperm viability; SPNR = Sperm normalcy

Table 2

Percentage of total sperm cell abnormalities recorded in five bull breeds kept at National Artificial Insemination Centre

Breed	Normal (%)	Abnormal head (%)	Detached head (%)	Proximal cytoplasmic droplets (%)	Bent midpieces (%)	Bent tail (%)	Coiled tail (%)
Friesian	86.56±1.27^{ab}	13.44. ±1.27^{ab}	13.08±1.68^{a}	0.04±0.04^{a}	12.38±1.65^{a}	0.08±0.07^{a}	0.02±0.02^{a}
Ayrshire	87.68±1.47^{a}	12.32±1.47^{a}	10.85±1.68^{ab}	0.11±0.04^{a}	10.13±1.65^{ab}	0.19±0.07^{a}	0.02±0.02^{a}
Jersey	83.96±1.63^{ab}	16.04±1.63^{ab}	14.58±1.94^{ab}	0.12±0.05^{a}	14.02±1.91^{ab}	0.08±0.08^{a}	0.07±0.02^{a}
Simmental	90.20±1.92^{ab}	9.8±1.92^{ab}	8.34±2.4^{ab}	0.08±0.06^{a}	7.74±2.33^{ab}	0.08±0.09^{a}	0.04±0.03^{a}
Boran	93.70±1.86^{ab}	6.30±1.86^{ab}	7.03±2.37^{ab}	0.04±0.04^{a}	6.53±2.33^{ab}	0.06±0.09^{a}	0.03±0.03^{a}

^{ab} *Means on the same column not sharing the common superscripts for each quality trait differ significantly (p<0.05). Values in the table represent the mean ± SE*

Influence of collection period on semen quality

We further analyzed our data to see the influence of the period (timing) of collection on semen parameters (Table 3) and revealed a significant difference *(p<0.05)* in the volume of semen collected during morning hours (5243.64±252.87mm^3) compared to evening hours (3970.31±252.87mm^3). Similarly, the average concentration and PM were 1266.82±94.05 M/ml, 63.27±1.60 percent during morning collection, and 1133.08±94.05 M/ml and 62.30±1.60 percent during evening collection, although the variation was not statistically significant. Concerning TM, viability, and morphological characteristics, better values were obtained during morning collection with statistical significance at *P<0.01* except for morphological defects.

Table 3

Comparison of fresh semen quality traits at different collection periods from bulls kept at National Artificial Insemination Centre

	Collection period	
Parameters	Morning (AM)	Evening (PM)
Volume (mm^3)	5243.64±252.87^a	3970.31±252.87^b
Concentration (M/ml)	1266.82±94.05^a	1133.08±94.05^a
Progressive motility (%)	63.27±1.6^a	62.30±1.6^a
Total motility (%)	87.01±1.51^a	78.55±1.51^b
Sperm viability (% live)	89.52±1.33^a	85.24±24^b
Sperm morphology (% normal)	89.12±1.03^a	87.71±1.03^a

^{ab}Means across the row not sharing the common superscripts, for each quality trait differ significantly *(p<0.05).* Values in the table represent the mean ± SEM

The influence of ejaculate number and age of the bulls

Furthermore, we checked the influence of the ejaculate number and age of the bulls on the quality of semen in this study. We found that the values are comparable (no significance) between ejaculates for almost all assessed parameters except for PM and TM which were slightly different at *P<0.05* (Table 4).

Table 4

Comparison of fresh semen quality traits in different ejaculates from bulls kept at National Artificial Insemination Centre

	Ejaculate number	
Parameters	First ejaculate	Second ejaculate
VOL (mm^3)	5590.76±106.78^a	5536.52±106.78^a
Concentration (M/ml)	1635.83±62.20^a	1773.54±62.20^a
Progressive motility (%)	60.16±0.74^a	62.41±0.74^b
Total motility (%)	81.77±0.64^a	84.67±0.64^b
Sperm viability (% live)	84.99±1.70^a	87.42±1.14^a
Sperm morphology (% normal)	89.87±1.32^a	88.13±0.88^a

^{ab}*Means across the row not sharing the common superscripts, for each quality trait differ significantly (p<0.05). Values in the table represent the mean ± SEM*

Concerning the age of the bulls and its influence on quality, we observed that only slight variation can be found among bulls of different ages across various parameters as detailed in Table 5. However, the mid-age (mature) group of 3-5 years deviated from other groups (the younger <3 years and older >5 years) having higher values in VOL, PM, TM and SPVI (55.66.17mm^3, 67.35%, 86.83%, and 89.75%) respectively.

Table 5

Comparison of fresh semen quality traits in different age groups from bulls kept at National Artificial Insemination Centre

	Age groups		
Parameters	< 3 years (n = 4)	3 – 5 years (n = 6)	> 5 years (n = 5)
Volume (mm^3)	3332.73±323.8^{b}	5566.17±494.92^{a}	4922.03±216.20^{ab}
Concentration (M/ml)	1199.84±120.43^{a}	1136.54±184.06^{a}	1263.46±8.40^{a}
Progressive motility (%)	59.76±2.05^{b}	67.35±3.14^{a}	61.25±1.37^{ab}
Total motility (%)	79.76±1.93^{b}	86.83±2.95^{a}	82.32±1.29^{ab}
Sperm viability (% live)	84.99±1.70^{a}	89.75±2.6^{a}	87.42±1.14^{a}
Sperm morphology (% normal)	89.87±1.32^{ab}	85.50±2.02^{b}	89.88±0.88^{ab}

^{ab}Means across the row not sharing the common superscripts, for each quality trait differ significantly *($p<0.05$).* Values in the table represent the mean ± SEM

Discussion

Normal reproduction in male animals is measured by the ability to produce semen with normal and adequate spermatozoa, as well as the desire and ability to mate. Hence, the assessment of freshly collected bull semen before use is of great importance in the AI industry as far as the semen of high-quality concerns and the need to achieve optimum reproductive efficiency. Semen quality is assessed by various parameters including common volume, concentration, percentage PM, percentage TM, viability, normalcy, and abnormalities. For this reason, we evaluated several parameters including the volume of ejaculate, the concentration of sperm, viability, morphology, and motility of sperm which are recommended as good indicators of semen quality. In this study, the values for these parameters were within the range of the value used by AI centers in many countries (Fordyce *et al*., 2006; Hirwa et al., 2017; Nagata *et al*., 2019; Kefelegn *et al*., 2021).

However, we have realized in this study that semen quality can be variable based on the breed of bulls from which semen was collected and variation can be to volume, TM, semen viability and morphology. Breed differences in semen quality and attributes are a subject of attention confirmed by several scientists elsewhere (Tohura *et al*., 2018; Alemayehu and Tena, 2015; Dasinaa and Pagthinathan, 2015; Kefelegn *et al*., 2021). In addition, the variation can be based on various parameters describing semen quality. For example, in a study conducted by Hirwa *et al*., (2017), a higher volume of semen was obtained in Friesian bulls compared to Jersey. In the present study, the Simmental bulls seem to be better in terms of semen volume compared to the rest of the bulls due to their behavior of abstaining/taking a long time to donate semen resulting in the accumulation of seminal fluid hence higher semen volume. Although all values for semen volume were within the recommended rates, higher volumes are better predictors of semen quality and significantly correlated with fertility of bovine semen (Fiaz, 2010). Similarly, variation has been observed among bulls on sperm concentration by various researchers. For instance, Adamczyk *et al*., (2013) and Alemayehu and Tena (2015) at different places reported significant differences in semen concentrations between different breeds. A lack of significant variation has been reported as well by several researchers including Boujenane and Boussaq (2014). Likewise, sperm PM can differ among bulls, between the number of ejaculates (e.g., ejaculate 1 and 2), and between periods of collection such as morning and evening as was in this study. Regarding TM breed differences as observed have also been reported elsewhere (Asad *et al*., 2014; Tohura *et al*., 2018). Values for two parameters (spermatozoa concentration and PM) were not statistically different among the breeds.

The viability (percentage of live sperms) and normalcy (percentage of normal sperm cells) in the ejaculates are parameters of significant importance as far as AI is concerned and this is because the number of viable spermatozoa deposited in the female reproductive tract influences the fertilizing ability of the cow (Pereira *et al*., 2010). It is good to note that values reported for these parameters in this study were within the

range for bulls used in AI services elsewhere (Oosthuizen, 2021). We also notify AI stakeholders that sperm viability can vary among bulls of different breeds and collection periods e.g morning and evening whereas the percentage of normal sperms in ejaculate can differ due to the age and breed of the bulls (Lemma, 2011; Alemayehu and Tena, 2015). We have established that the first ejaculate had a greater semen volume with lower sperm concentration per ejaculate as compared to the second ejaculate. Varying results can be obtained in literature, see for example Dukelow *et al*. (1960); Murphy *et al.,* (2018) and Taaffe *et al*., (2022). Importantly, Fuerst-Waltl, *et al.* (2006) added that the successive increase in ejaculate number after the second, the volume and sperm number gradually start to decrease, a fact which was also confirmed by Hafs *et al*., (1959) and contrasted by Mukesh *et al*., (2011). In our study as per ejaculate number, no differences were found regarding semen viability and morphology which were parallel to report provided by Hafs *et al*., (1959).

Another non-genetic factor that needs to be considered in AI, particularly for semen is the age of the bull and several scientific reports show various opinions of researchers in the AI industry. Two reports, Mukesh *et al*., (2011) and Tohura *et al*., (2018) showed that the volume of semen increases up to the age of 4 to 5 years and then starts to decrease as age advances e.g., 6 years. This is associated with increasing the size of the testicles, nutritional status, geographic locations, season of the year, method of semen collection, and handling of bulls during collection (Tegegn *et al*., 1992; Mirza and Zahid, 2003 and Hafez, 2013). A few researchers e.g., Hirwa *et al*., (2017) and Kefelegn *et al*., (2021) have reported contrasting findings that the volume increased as age increases. Other semen quality parameters are influenced differently by bulls of different age for instance a study by Dhami and Kodagali (1988) explained that PM and TM increase with age up to 5 years and starts to decrease as the age increases above 5 years. This increase could be due to high sperm concentration low sperm abnormalities, and management conditions as stated by Koonjaenak *et al*., (2007). Moreover, the decrease in semen attributed due to increasing age maybe due to decreasing function of the post-testicular glands and decrease in epididymis function and a change in the function of sperm mitochondria which is very important for sperm, although motility decreases with age in bulls, still meets the minimum standard requirements for AI (Sloter *et al*., 2006). Furthermore, Hirwa *et al.,* (2017) agreed that age affected the number of live spermatozoa as bulls aged 3-5 years had a higher number of live spermatozoa compared to those aged above 5 years. About sperm viability, Ahmad *et al*., (2003); Mukesh *et al*., (2011); Tohura *et al*. (2018); Pardede *et al*., (2020) and Tohura *et al*., (2018) reported that it is a quality parameter that is also affected differently by bulls of different age. Concerning the percentage of normal spermatozoa Ahmad *et al*., (2003); Vilakazi (2003); Mostari *et al*., (2005) and Tohura *et al*., (2018) showed that bull's aged3-4yearsexhibited a higher percentage than bulls older than 6 years and bulls younger than 3 years of age. Furthermore, major sperm defects including pyriform, knobbed acrosomes, mid-piece reflexes, and Dag defects can occur and are associated with age differences among the bulls. This is also attributed to physiological changes that occur as bulls grow to sexual maturity, fat deposition in the scrotum, which increases with age and can lower the efficiency of scrotal thermo-regulation by reducing the amount of heat that can be radiated from the scrotal neck, hence inferior semen quality will be produced in old bulls (Barth and Oko, 1989; Kommisrud and Berg, 1996; Coulter *et al*., 1997). With this discussion it's therefore a call for researcher, farmers, artificial inseminators and stakeholders of the AI industry in the country broad to trust that semen produced from the bulls raised at NAIC Arusha is of good quality with exclusion of other external factors.

Conclusion

It can be concluded that the proportional of total motility and sperm viability among five bull breeds were significant. Better semen qualities were recorded for the bulls of middle age 3 – 5years. The quality parameters (volume, progressive motility, total motility and sperm viability) except for concentration and sperm morphology showed positive increase in value with increasing age of the bulls up to five years and started to decrease at the age above five years. Jersey and Simmental bulls had semen with higher quality value $P<0.05$ compared to other breeds, although all bull breeds were observed to have good quality

semen which meets standards. Semen quality was low at evening time while the morning was ideal for semen collection due to cool condition and high libido exhibited by bulls. Furthermore, our results revealed that breed, age period of semen production, and ejaculate number have a significant effect on bovine semen characteristics. Hence the bull breeds aged 3-5 years should be promoted at the center and all two ejaculates at morning time are ideal for processing. We recommend in addition that although fresh semen quality has no problem and be used for further processing, NAIC should put higher priority in dairy production system by considering the relationship between nutrition, reproductive physiology and management of bulls. Finally further studies are highly recommended on this regard.

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Conflict of interest

The authors declare that there is no conflict of interests.

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