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Breeding Soundness Examination of Mpwapwa Breed Bulls: Suitability for Use as Natural Service and Artificial Insemination Sires

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Abstract

Breeding soundness examination, semen examination and sperm DNA fragmentation were assessed in Mpwapwa breed bulls to determine their suitability for use as natural service or AI sires. Based on the available research facility, 53 of the 120 Mpwapwa bulls at the Tanzania Livestock Research Institute (TALIRI) were examined. Bulls were 28 - 49 months old (Mean: 40.7, SD: 13.6 months) and BCS 4 (1 - 5 scale). Mean scrotal circumference was 27.5 cm (Range: 24 - 33 cm, SD: 1.9) cm, and was unrelated to age ($p > 0.50$). Semen was collected by electro ejaculation. Six bulls produced only seminal plasma. Mean volume of the remaining ejaculates was 5.5 mL (range: 1.9 - 14.9 mL, SD: 1.9 mL). Mean ejaculate density was 303×10^6 sperm/mL (range: 57 - 966, SD: 258): 31 bulls produced ejaculates with $\leq 400 \times 10^6$ sperm/mL and 8 bulls ejaculates with $\geq 700 \times 10^6$ sperm/mL. The mode score for mass motility (4-point scale: −, +, ++, +++) was ++ (24 bulls: 51%), 23% of bulls (11) were scored as +++ and 12 (26%) as +. Most (41/47: 87.2%) bulls had ≥70% motile sperm, but only 19 bulls had ≥80% motile sperm. Few (4/47, 8.5%) bulls had <70% morphologically normal sperm. Most bulls had few fragmented sperm (unfragmented mean: 94.7%, mode: 100%). The proportion of unfragmented sperm was unrelated to progressive motility or bull age. Eight bulls had semen (density, morphology and motility) results that met established criteria for use in AI. A total of 15 bulls with ejaculate density $\geq 300 \times 10^6$ sperm/mL and normal morphology ≥80% would be suitable for use as natural service sires for low-intensity breeding.

Subject Areas

Bull Fertility and Artificial Insemination

Keywords

Artificial Insemination Breeding Programme, Breeding Soundness Examination, DNA Fragmentation, Mpwapwa Breed Bulls Semen Quality

1. Introduction

The Mpwapwa breed of *B. indicus* cattle was initially developed during the 1940s as a locally adapted breed to improve the quality of beef cattle in Tanzania [1]. Mpwapwa cattle were initially disseminated by direct sales of breeding cows and bulls and later by artificial insemination (AI). However, AI services in central Tanzania largely ceased in 1973, when AI services were transferred to the National Artificial Insemination Centre (NAIC) [2], resulting in a marked attenuation of the Mpwapwa dissemination programme. Consequently, on-farm breeding of Mpwapwa cattle has become significantly limited, to the extent that, even when they are bred, it is largely through natural service by local non-selected bulls. Genetic improvement has therefore largely ceased [3]-[5], except at the Tanzania Livestock Research Institute (TALIRI), where a herd of Mpwapwa cattle has been maintained. The Mpwapwa bulls at TALIRI have undergone genetic selection for conformation, growth rate and feed conversion efficiency during the period from 1973, so the herd represents a potential nucleus herd which could form the basis of an AI service for smallholder beef farmers, although, there are has been no selection of bulls in the TALIRI herd for breeding soundness or fertility traits.

In 2018, TALIRI re-equipped its animal biotechnology laboratory so that it could restart an AI programme for local beef producers. The focus of that programme is to, initially at least, increase the use of Mpwapwa genetics within local herds. Two potential routes have been identified: firstly, by the reinstatement of an AI programme and, secondly, by supplying bulls from TALIRI directly to beef farmers in the central zone of Tanzania. An AI service would require appropriate infrastructure at both the TALIRI stud and for the distribution of semen across the region, whereas direct sale of bulls would require much less investment. Regardless, any dissemination of Mpwapwa genetics requires that bulls have semen of adequate quality (i.e. in terms of potential fertilizing capacity) and quantity [6] [7]. Standards for AI sires are generally higher than those for natural-service sires, but in either case, elimination of animals likely to be of low fertility and promotion of animals likely to be of high/adequate fertility is essential. As the potential fertility of Mpwapwa bulls has not been assessed (at least since 1973), doing so is an essential prerequisite for the re-establishment of an AI service and/or direct bull sales.

There are several, broadly similar, bull soundness examination (BSE) protocols for assessing bull fertility (e.g. [8] [9]). Such BSE schemes provide simple, repeatable and unambiguous procedures for bull fertility evaluation. Under the Australian Bull Check scheme [9], there are four assessment components: (i) general physical examination (*i.e.* structural and reproductive soundness); (ii) examination of the scrotum (scrotal circumference, testes, epididymides); (iii) assessment of libido and mating ability; and (iv) collection and assessment of semen (including sperm morphology). The criteria of the American Society for Theriogenology [8]-[11] particularly focus on scrotal circumference, percentage of motile sperm and percentage of sperm with normal morphology; with bulls being classified as either satisfactory (potentially fertile), deferred, or unsatisfactory (potentially subfertile/sterile). The evaluation of bovine semen during a BSE is generally confined to motility, concentration and morphology [12]-[16], although additional functional tests are sometimes included in the evaluation of semen for use in high-intensity AI services. Whilst it is generally recognised that meeting the minimum acceptance criteria does not correlate highly with conception rates, bulls that do not meet the minimum criteria are unlikely to perform adequately, so can be screened out of use in the programme.

Because the standard BSE does not directly predict bulls' fertility [12] [13] [17] [18], additional methods are sometimes used to try to assess fertility more directly. The inclusion of emerging molecular technologies in the BSE evaluation programme has therefore been suggested as a means of improving the efficiency of predicting sperm fertility [18] [19]. One such method is the assessment of DNA fragmentation. Sperm DNA fragmentation has been identified in recent years as a major cause of human male infertility with causal effects on fragmentation ascribed to environmental insults [20] [21] and advancing age [22]-[24]. Fragmentation has also been identified as a cause of impaired fertility in bulls [25] [26]. Estimation of the degree of DNA fragmentation may therefore be useful for identifying bulls in AI programmes that are at risk of having inherently impaired fertility, especially in a population that has not been subjected to selection for fertility over a long period of time.

The purpose of this study was therefore to undertake breeding soundness and semen examination of the stud of Mpwapwa bulls in the TALIRI nucleus herd, to determine the suitability of those bulls as natural service sires and as AI semen donors. DNA fragmentation was also examined, as it may provide additional information regarding the likely fertility of the bulls. Further, as the opportunities for studying sperm DNA fragmentation in an entirely unselected population of bulls are few and far between, the relationship of DNA fragmentation with classic semen examination was of particular interest.

2. Materials and Methods

Breeding soundness examination of Mpwapwa bulls was undertaken at the TALIRI research centre in the Dodoma region of Tanzania. The climate of Mpwapwa is subtropical but is modified by altitude. There are two breeding seasons for cattle: the first between March and May (at the end of the rainy season) and the second between September and November (at the end of the dry season). All animal-related manipulations received Livestock Research Ethical Clearance from the Tanzanian Livestock Research Institute (TALIRI) (12/02/2021) prior to the start of the study. All bulls came from the TALIRI Mpwapwa research herd. There were 120 mature (≥2 years old) Mpwapwa breed bulls available for selection. Bulls were weighed and body condition scored (1 - 5 scale; [27]) and 53 bulls selected for the BSE study based on available research facility. The 53 selected bulls were treated with 5 mg/kg levamisole (Levamizole, Eagle Vet. Tech Co. Kenya) for endoparasites control. For a period of 4-weeks before examination, all animals were given access to unrestricted grazing (stocking rate 0.36 livestock units/ha) in 15 ha paddocks and were trough-fed 0.6 kg/bull/day of a compound ration made from 600 g/kg maize bran, 390 g/kg sunflower seed cake and 10 g/kg salt, and had access to mineral lick blocks (Farmers Centres Ltd, Tanzania) at an allocation rate of 400 g/bull/week. Bulls were dipped weekly in a bath containing 100 g/L alphacypermethrin (Paranex, Farm Base Ltd, Tanzania) for ectoparasites control. BCS (1 - 5 scale; [27]) was assessed and weight measured as each bull left the crush after the 4-week management period.

2.1. Breeding Soundness Examination

Ten or 11 bulls were examined per day over a period of 5 days. The BSE component was based on the procedures outlined by [8]-[10] [28]. Bulls were observed walking on a hard surface to check for lameness and locomotion problems that could affect mating ability. They were then confined in a crush for external and transrectal examination of the genital system, with the scrotum (testes), epididymis and spermatic cords being examined to see if they were non-painful, smooth and moved freely. The prepuce and penis were then checked for thickening, adhesions and the presence of discharge. This examination was followed by measurement of scrotal circumference (SC).

Semen was collected by electroejaculation (Ejakulator, Minitube GmbH, Germany) into an artificial vagina cone. Ejaculates were maintained at ambient temperature (~30˚C) and taken to the laboratory within 1 to 2 minutes of collection. The volume of the ejaculate was recorded, its colour assessed and the absence of blood and urine staining confirmed. Concentration was measured by diluting 10 μL semen with 1 mL of sodium chloride solution (0.9% w/v) in a cuvette and placing into a previously-calibrated spectrophotometer (Accuread Photometer, Biochrom Ltd, USA) for absorbance reading. Mass motility was examined in undiluted semen, and individual motility examined after 1:1 dilution in Optixcell diluent (IMV, L'Aigle, France) using phase-contrast microscopy (MBL2000 Kruss Optronic GmbH, Germany) at ×10 (mass) and ×100 - ×400 (individual) magnifications. Smears were made of undiluted semen and were stained with eosin-nigrosin for morphology examination: 100 sperm were randomly counted at x1000 magnification. Sperm with abnormal morphology were classified by site and, according to the criteria of [29], into major/minor defects.

2.2. DNA Fragmentation

Sperm DNA fragmentation experiment was undertaken as a joint venture with NAIC in Arusha. Fragmentation was assessed using the Sperm Chromatin Dispersion (SCD) (GoldCyto DNA: Microptic, Barcelona, Spain) method, according to the manufacturer's instructions. The principle of the method is that sperm with DNA fragmentation will fail to produce the halo characteristic of the dispersed DNA loops that are present in sperm that have non-fragmented DNA, after acid denaturation and removal of nuclear proteins [30] [31]. For this process, intact, unfixed sperm are immersed in an inert agarose microgel on a pre-treated slide. An initial acid treatment is then used to denature any fragmented DNA present in those sperm. This is followed by a lysing solution which removes most of the nuclear proteins and, in the absence of DNA breakage, produces nucleoids with large halos of spreading DNA loops that emerge from a central core. In contrast, the nucleoids from sperm with fragmented DNA either do not show a dispersion halo or have a small halo only. Sperm without a halo or a small halo are classified as having DNA fragmentation, whereas those with larger halos are classified as normal. Degraded sperm are not counted.

2.3. Statistical Methods

Descriptive data were compiled and presented as box-plots or bar charts. Where appropriate, data were compared using Spearman's rank correlation or univariate ANOVA.

3. Results

All bulls were assessed as having adequate conformation and locomotor action. No bulls were observed as having gross lesions of the external or internal genitalia. SC ranged from: 24 - 33 cm (mean 27.5 cm, SD: 1.9 cm) (**Figure 1**), and was unrelated to age ($\rho = 0.24$; $p = 0.19$). Furthermore, when bulls were separated into those ≤ 36 months and those ≥ 36 months, mean SC for the two groups were similar (27.1, SD: 1.6 cm *versus* 27.8, SD: 2.0 cm, respectively).

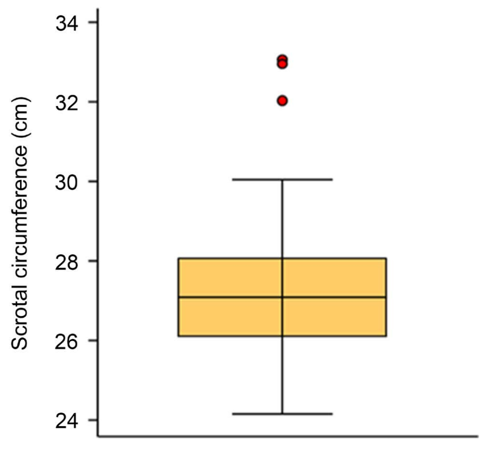

Figure 1. Box plot of scrotal circumference of 53 Mpwapwa bulls.

Semen was successfully collected from 47/53 bulls; the remaining six animals produced only seminal plasma. Mean ejaculate volume over all bulls was 5.5 mL (range: 1.9 - 14.9 mL, SD: 2.6 mL). When bulls that produced seminal plasma were excluded, there was no change in mean volume (mean: 5.5 mL, SD: 2.7 mL). Excluding bulls that produced only seminal plasma, mean ejaculate density was relatively low, at 303 × 10^6 sperm/mL (range: 57 - 966 × 10^6 sperm/mL, SD: 258 × 10^6 sperm/mL), with 31/47 (66%) bulls producing ejaculates with ≤400 × 10^6 sperm/mL. Eight bulls produced ejaculates with ≥700 × 10^6 sperm/mL. All other ejaculates were ≤600 × 10^6 sperm/mL. Ejaculate density data are shown in Figure 2. Ejaculate density was not correlated with SC or bull age (ρ = 0.2 and 0.01, respectively; p ≥ 0.177).

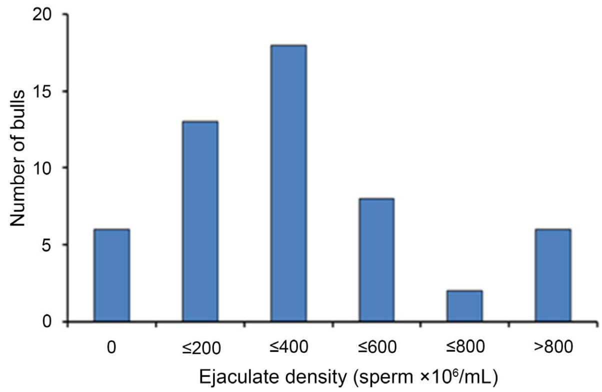

Figure 2. Ejaculates of different densities after electroejaculation of 53 Mpwapwa bulls.

Mass motility scores varied from + (12 bulls: 26%), ++ (24 bulls: 51%) to +++ (11 bulls: 23%). No bulls whose ejaculates contained sperm had zero (−) mass motility. Individual motility scores were generally ≥70%, with only 6/47 bulls having scores of ≤60% (Figure 3). However, only 19 bulls had ejaculates that contained ≥80% motile sperm. Proportion of progressively motile sperm was associated with mass motility scores (P<0.001) with bulls with +++ scores having higher numbers of motile sperm (85%; 95% confidence interval (CI) 79.1 - 90.9) than bulls with + scores (64.2%; 95% CI ± 58.6% - 69.8%) or ++ scores (74.4%; 95%CI ± 70.4% - 78.4%).

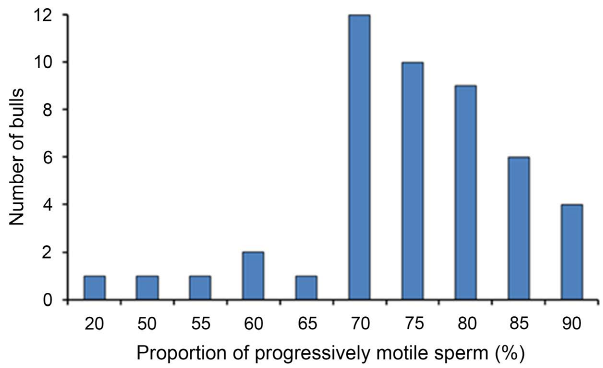

Figure 3. Proportions of progressively motile sperm in the ejaculates of Mpwapwa bulls. Data from 47 bulls as data excluded from the 6 bulls which produced aspermic ejaculates (see Figure 1).

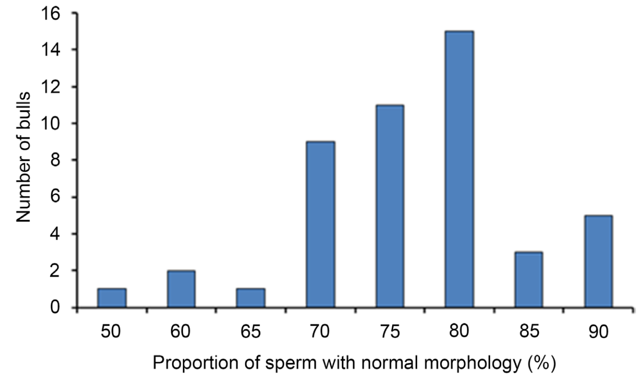

Figure 4. Proportions of sperm with normal morphology in ejaculates from 47 Mpwapwa bulls.

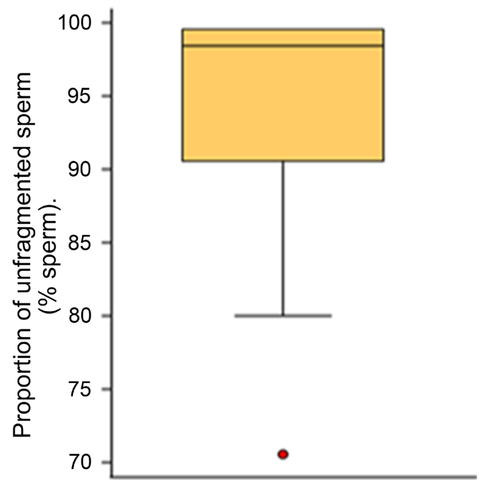

Figure 5. Distribution of the proportion of unfragmented sperm in ejaculates from 35 Mpwapwa bulls.

Interpretation **Figure 1** and **Figure 5**: The central box spans the quartiles, and the line in the box denotes the median. The line extends from the box (whiskers) to 1.5 times the interquartile range. Observations more than 1.5 times the interquartile range from the median are plotted individually as possible outliers (asterisks).

Proportions of morphologically normal sperm are shown in **Figure 4**. Only 4/47 (8.5%) of bulls had less than 70% morphologically normal sperm in their ejaculates. There were very limited differences in the site of abnormalities or in the distribution of major/minor defects between bulls. Results for the proportion of undegraded sperm (i.e. no DNA fragmentation) are shown in **Figure 5**. Most bulls clustered around the mean of 94.7% (SD: 6.8, Mode: 100%). The proportion of unfragmented sperm was unrelated to bull age or to initial progressive motility of the ejaculate ($\rho = 0.12$ and −0.09, respectively; $p > 0.5$).

Overall assessment

Four bulls met the criteria of ≥800 $\times 10^6$ sperm/mL ejaculate density, at least ++ on mass activity, ≥80% progressive motility and ≥70% morphologically normal sperm which are generally recommended when a bull is to be used for AI [8] [9] [12]. Four other bulls met three of these four criteria and were close to meeting the fourth. The results of these eight bulls are shown in **Table 1**. Inclusion of an SC target of ≥28 cm [32] reduced the number of bulls meeting all criteria to two (14983 and 14911).

Table 1. Scrotal circumference and semen quality data from eight Mpwapwa bulls that met/almost met the minimum criteria for use in an AI service.

ID	SC (cm)	Volume (mL)	Mass activity	Individual motility (%)	Morphology (% normal)	Sperm/mL ($\times 10^6$)	Fragmentation (%)
14967	28	5.8	+++	75	80	966	6.7
14983	29	2.8	+++	80	85	896	0
14901	25	3	+++	90	80	873	4.6
13128	29	8	++	75	75	829	8.8
14804	26	5.5	+++	85	75	820	0
14911	29	8	+++	90	80	815	5.7
14841	33	6	++	80	80	746	9.1
14842	29	8	+++	85	80	734	12

SC: scrotal circumference, Mass activity: +++ = vigorous swirling, ++ = moderate swirling; Box colour: Light green: result meets the minimum criteria for use in an AI service; Buff: result is marginal for use in an AI service. Box colour for individual bulls is light green if all criteria meet minimum standards and buff if one or more results is marginal.

Fifteen bulls (numbers 13128, 14967, 14975, 14980, 14983, 14966, 14922, 14924, 14901, 14911, 14841, 14842, 14858, 14791 and 14804) had ejaculates of density ≥300 × 10^6 sperm/mL and ≥70% morphologically normal sperm. These bulls meet the criteria for *B. indicus* bulls for widespread use as natural service sires.

4. Discussion

This is the first large-scale study of the breeding characteristics of Mpwapwa bulls that has been reported since the breed was developed during the 1940s. Although the breed has been maintained for dissemination and conservation, it has only been subject to a relatively low-level of selection for production and has had no selection for breeding. Several studies have been conducted on the female reproductive performance of Tanzania Short-horned Zebu (TSZ) cattle (e.g. [33] [34]), but studies on breeding bulls of Tanzanian origin are rare.

The routine use of BSE in bulls has a significant impact on the reproductive performance of herds, particularly in terms of conception/pregnancy rate. Consistently higher pregnancy rates are reported from bulls that are classified as satisfactory compared to bulls not tested or which failed to score a satisfactory standard [35]. An early study of the outcomes of BSE in *Bos taurus* in the USA reported 75%, 52% and 12% pregnancy rates using single-sire bulls classified as satisfactory, questionable and unsatisfactory respectively [36]. Similarly, an improvement in pregnancy rate was reported in cows bred to bulls selected for semen quality *versus* cows bred to unselected bulls [37].

After eliminating bulls that have physical incapacity for breeding (e.g. lame, penile lesions), scrotal circumference and semen quality are regarded as being the most important aspects of the BSE. In the present study, the mean SC of Mpwapwa bulls was 27.5 cm (SD: 1.9 cm). The recommended minimum SC for *B. indicus* and derived crosses is 28 cm [32] for 2-year-old bulls kept in a herd under semi-arid agro-ecological conditions. In the present study only 27/53 bulls (51%) met this minimum, whilst a further 10 bulls (19%) had SC of 27 cm. Conversely, 20 bulls (37%) had SC of ≤26 cm. Thus, only about half of the bulls met the criterion of SC ≥ 28 cm, but 79% of bulls were within 1 cm of the minimum. Whether the minimum of 28 cm [32] is the correct standard to apply to the Mpwapwa breed therefore requires some careful consideration. Firstly, it was clear that semen quality (in terms of total sperm/ejaculate and proportion of sperm with normal morphology) was largely unrelated to SC, and certainly could not be divided into groups that were defined by having SC ≥ 28 or <28 cm. Of the bulls that were deemed to have produced semen that was of acceptable quality for AI (**Table 1**), 2/8 had SC of <28 cm, but, despite failing to achieve the minimum SC, these bulls produced semen of acceptable quality. This weak correlation between SC and sperm production is somewhat at variance with the literature in which SC and capacity for sperm production generally have a positive correlation (e.g. [38]-[40]). In a study of a similar breed of low-bodyweight *B. indicus* bulls in Costa Rica, a positive relationship between SC and sperm production, and semen quality was reported [41]. Perhaps the relationship between SC and semen density in the present study was affected by the generally low ejaculate densities, or, more likely, reflects the scatter that is inherent in an unselected breed.

Most bulls in the present study were between 2 and 4.5 years: a period that is associated [38]-[40] with the maximum growth rate of the testes. Hence, some variation in the relationship between SC and ejaculate density in the present study might possibly be accounted for in terms of bull age. On the other hand, the lack of evidence for a net increase in either SC or ejaculate density between the young and older bulls does not support this suggestion. More extensive tracking of SC in bulls between 2 and 4 years of age might show in better detail how the SC of Mpwapwa breed bulls' changes with time: for example, as a low body weight breed, it might be that SC has already reached its plateau at ~2.5 years, unlike the 4+ years of heavy-framed animals. More general studies of *B. indicus* and *B. taurus* bulls have established that different breeds of bull show significant variation of SC between breeds and strains within breeds. These, may be intrinsic characteristics of the breeds themselves, or may be a consequence of differences in mature body weight [42]-[44]. Thus, the relatively high target for SC of *B. indicus* bulls in the Australian BSE recommendations [32], may be a reflection that they were based on improved *B. indicus*, which are typically both larger framed and selected for breeding, rather than the relatively unimproved animals that are common in the *B. indicus* strains of East Africa, South Asia and South-East Asia. Evidence that this may be the case comes from a study of TSZ breeding bulls [45], which found SC ranging from 24 to 34 cm, which was similar to the 25 to 33 cm reported in the Mpwapwa bulls included in the present study. Scrotal circumference may also be limited by nutritional circumstances during the rearing period: [41] reported that SC was smaller in extensively managed *B. indicus* bulls with low BCS than in bulls with normal BCS. Likewise, [46] found that mature SC of *B. indicus* bulls raised in dry-tropical conditions was limited by the adverse climatic and nutritional circumstances under which bulls had been raised, and [45] reported in extensively reared TSZ bulls that SC was correlated with heart girth (i.e. body weight/size). That study, however, unlike the present observations of Mpwapwa bulls, found that bulls with larger SC tended to produce ejaculates with higher density and volume than bulls with smaller SC.

DNA fragmentation studies of bovine sperm are relatively few, with most coming from *B. taurus*. Studies of *B. indicus* are relatively rare, and studies of the small-framed East African Zebu cattle are rare indeed. The scarcity of such studies is a reflection of the costliness of undertaking DNA fragmentation studies, which precludes their routine use in standard operating procedures for semen production in most commercial AI centres. The results from the current study showed that there was no relationship between the proportion of unfragmented sperm and either the age of the bull or the initial progressive motility of the ejaculate. There were, however, two bulls in which the proportion of unfragmented sperm was well below 85%, so these animals would be worth further investigation. As, in general, fragmentation increases with age, the relatively young ages of the bulls in the present study may have precluded them from having yet having many damaged sperm. Older bulls, such as dairy bulls that have been through a progeny-testing cycle are therefore more likely to show fragmentation than the young animals in the present study [47]-[49].

In conclusion, this study was undertaken as no systematic assessment of the breeding soundness of the Mpwapwa has ever been previously carried out. The results show that there is a great deal of bull-to-bull variation in BSE and semen quality results; although most bulls produce semen that would be adequate for use in the natural service of small herds, and at least a proportion of the bulls produce semen that would be acceptable for use in AI. The results which were obtained in this study are quite similar to the results of other BSE studies done on B. indicus bulls globally, particularly the smaller-framed breeds of East Africa and South East Asia. The findings from the sperm DNA fragmentation study indicate that sperm DNA fragmentation was unlikely to be of significance in Mpwapwa bulls. Opportunities exist for more thorough characterisation of the growth profile of SC over the period between puberty and maturity, and to determine whether there is a relationship between mature body size and plateau SC values. Effects of optimising the plane of nutrition during the rearing period upon mature SC would also be worth studying. Nevertheless, whilst there are still further avenues to be explored for enhancing the fertility of Mpwapwa, this study has shown that at least some bulls produce semen that could be used in AI, and that BSE should be able to eliminate sires that are unlikely to achieve adequate fertility even in low-intensity natural-service programmes.

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Conflicts of Interest

Authors do not have any conflict of interest.

References

[1] Chawala, A.R., Banos, G., Komwihangilo, D.M., Peters, A. and Chagunda, M.G.G. (2017) Phenotypic and Genetic Parameters for Selected Production and Reproduction Traits of Mpwapwa Cattle in Low-Input Production Systems. *South African Journal of Animal Science*, **47**, 307-319. https://doi.org/10.4314/sajas.v47i3.7

[2] Mejooli, A.S.M. (1977) The Future Role of Artificial Insemination (AI) in Cattle Improvement in Tanzania. *Tanzania Society of Animal Production*, **4**, 119-160.

[3] MLFD (2011) The Tanzania Dairy Industry: Status, Opportunities and Prospects. *7th African Dairy Conference and Exhibition*, Dar es Salaam, 25-27 May 2011, 22 pp.

[4] Katjiuongua, H. and Nelgen, S. (2014) Tanzania Smallholder Dairy Value Chain Development: Situation Analysis and Trends. International Livestock Research Institute. https://cgspace.cgiar.org/bitstream/handle/10568/68513//PR_Tanzania.pdf

[5] Ogutu, C., Kurwijila, L. and Omore, A. (2014) Review of Success and Failures of Dairy Value Chain Development Interventions in Tanzania. International Livestock Research Institute.

[6] Parkinson, T.J. (2004) Evaluation of Fertility and Infertility in Natural Service Bulls. *The Veterinary Journal*, **168**, 215-229. https://doi.org/10.1016/j.tvjl.2003.10.017

[7] Barth, A.D. (2018) Review: The Use of Bull Breeding Soundness Evaluation to Identify Subfertile and Infertile Bulls. *Animal*, **12**, S158-S164. https://doi.org/10.1017/s1751731118000538

[8] Chenoweth, P.J., Spitzer, J.C. and Hopkins, F.M. (1992) A New Bull Breeding Soundness Evaluation Form. *Proceedings for the Annual Meeting of the Society for Theriogenology*, Montgomery, 63-70.

[9] Fordyce, G., Entwistle, K., Norman, S., Perry, V., Gardiner, B. and Fordyce, P. (2006) Standardising Bull Breeding Soundness Evaluations and Reporting in Australia. *Theriogenology*, **66**, 1140-1148. https://doi.org/10.1016/j.theriogenology.2006.03.009

[10] Kennedy, S.P., Spitzer, J.C., Hopkins, F.M., Higdon, H.L. and Bridges, W.C. (2002) Breeding Soundness Evaluations of 3648 Yearling Beef Bulls Using the 1993 Society for Theriogenology Guidelines. *Theriogenology*, **58**, 947-961.

https://doi.org/10.1016/s0093-691x(02)00911-1

[11] Koziol, J. and Armstrong, C.L. (2018) Society for Theriogenology Manual for Breeding Soundness of Bulls. The Society for Theriogenology.

[12] Graham, J.K. (2001) Assessment of Sperm Quality: A Flow Cytometric Approach. *Animal Reproduction Science*, **68**, 239-247. https://doi.org/10.1016/s0378-4320(01)00160-9.

[13] Rodriguez-Martinez, H. (2013) Semen Evaluation Techniques and Their Relationship with Fertility. *Animal Reproduction*, **10**, 148-159.

[14] Mocé, E. and Graham, J.K. (2008) *In Vitro* Evaluation of Sperm Quality. *Animal Reproduction Science*, **105**, 104-118. https://doi.org/10.1016/j.anireprosci.2007.11.016

[15] Chenoweth, P.J. and McPherson, F.J. (2016) Bull Breeding Soundness, Semen Evaluation and Cattle Productivity. *Animal Reproduction Science*, **169**, 32-36. https://doi.org/10.1016/j.anireprosci.2016.03.001

[16] Tanga, B.M., Qamar, A.Y., Raza, S., Bang, S., Fang, X., Yoon, K., *et al.* (2021) Semen Evaluation: Methodological Advancements in Sperm Quality-Specific Fertility Assessment—A Review. *Animal Bioscience*, **34**, 1253-1270. https://doi.org/10.5713/ab.21.0072

[17] Graham, E.F., Schmehl, M.K.L. and Nelson, D.S. (1980) Problems with Laboratory Assays. *Proceedings of the Eighth National Association of Animal Breeders Technical Conference on Artificial Insemination and Reproduction*, 59-66.

[18] Klein, E.K., Swegen, A., Gunn, A.J., Stephen, C.P., Aitken, R.J. and Gibb, Z. (2022) The Future of Assessing Bull Fertility: Can the 'Omics Fields Identify Usable Biomarkers? *Biology of Reproduction*, **106**, 854-864. https://doi.org/10.1093/biolre/ioac031

[19] Bailey, J., Morrier, A. and Cormier, N. (2003) Semen Cryopreservation: Successes and Persistent Problems in Farm Species. *Canadian Journal of Animal Science*, **83**, 393-401. https://doi.org/10.4141/a03-024

[20] Kumar, N. and Singh, A.K. (2022) Impact of Environmental Factors on Human Semen Quality and Male Fertility: A Narrative Review. *Environmental Sciences Europe*, **34**, Article No. 6. https://doi.org/10.1186/s12302-021-00585-w

[21] Szabó, A., Váncsa, S., Hegyi, P., Váradi, A., Forintos, A., Filipov, T., *et al.* (2023) Lifestyle-, Environmental-, and Additional Health Factors Associated with an Increased Sperm DNA Fragmentation: A Systematic Review and Meta-Analysis. *Reproductive Biology and Endocrinology*, **21**, Article No. 5. https://doi.org/10.1186/s12958-023-01054-0

[22] Evenson, D.P., Djira, G., Kasperson, K. and Christianson, J. (2020) Relationships between the Age of 25,445 Men Attending Infertility Clinics and Sperm Chromatin Structure Assay (SCSA®) Defined Sperm DNA and Chromatin Integrity. *Fertility and Sterility*, **114**, 311-320. https://doi.org/10.1016/j.fertnstert.2020.03.028

[23] Gill, K., Jakubik-Uljasz, J., Rosiak-Gill, A., Grabowska, M., Matuszewski, M. and Piasecka, M. (2020) Male Aging as a Causative Factor of Detrimental Changes in Human Conventional Semen Parameters and Sperm DNA Integrity. *The Aging Male*, **23**, 1321-1332. https://doi.org/10.1080/13685538.2020.1765330

[24] Ashapkin, V., Suvorov, A., Pilsner, J.R., Krawetz, S.A. and Sergeyev, O. (2022) Age-associated Epigenetic Changes in Mammalian Sperm: Implications for Offspring Health and Development. *Human Reproduction Update*, **29**, 24-44. https://doi.org/10.1093/humupd/dmac033

[25] Kumaresan, A., Johannisson, A., Al-Essawe, E.M. and Morrell, J.M. (2017) Sperm

Viability, Reactive Oxygen Species, and DNA Fragmentation Index Combined Can Discriminate between Above- and Below-Average Fertility Bulls. *Journal of Dairy Science*, **100**, 5824-5836. https://doi.org/10.3168/jds.2016-12484

[26] Boe-Hansen, G.B., Fortes, M.R.S. and Satake, N. (2018) Morphological Defects, Sperm DNA Integrity, and Protamination of Bovine Spermatozoa. *Andrology*, **6**, 627-633. https://doi.org/10.1111/andr.12486

[27] Nicholson, M.J. and Sayers, A.R. (1987) Repeatability, Reproducibility and Sequential Use of Condition Scoring of Bos Indicus Cattle. *Tropical Animal Health and Production*, **19**, 127-135. https://doi.org/10.1007/bf02239705

[28] Kastelic, J.P. and Thundathil, J.C. (2008) Breeding Soundness Evaluation and Semen Analysis for Predicting Bull Fertility. *Reproduction in Domestic Animals*, **43**, 368-373. https://doi.org/10.1111/j.1439-0531.2008.01186.x

[29] Blom, E. (1983) Sperm Morphology with Reference to Bull Infertility. *First All-India Symposium on Animal Reproduction*, Ludhiana, 61-81.

[30] Fernández, J.L., Muriel, L., Rivero, M.T., Goyanes, V., Vazquez, R. and Alvarez, J.G. (2003) The Sperm Chromatin Dispersion Test: A Simple Method for the Determination of Sperm DNA Fragmentation. *Journal of Andrology*, **24**, 59-66. https://doi.org/10.1002/j.1939-4640.2003.tb02641.x

[31] Fernández, J.L., Muriel, L., Goyanes, V., Segrelles, E., Gosálvez, J., Enciso, M., *et al*. (2005) Simple Determination of Human Sperm DNA Fragmentation with an Improved Sperm Chromatin Dispersion Test. *Fertility and Sterility*, **84**, 833-842. https://doi.org/10.1016/j.fertnstert.2004.11.089

[32] McGowan, M.R., Bertram, J.D., Fordyce, G., Fitzpatrick, L.A., Miller, R.G., Jayawardhana, G.A., *et al*. (2002) Bull Selection and Use in Northern Australia. *Animal Reproduction Science*, **71**, 25-37. https://doi.org/10.1016/s0378-4320(02)00023-4

[33] Kanuya, N.L., Matiko, M.K., Kessy, B.M., Mgongo, F.O., Ropstad, E. and Reksen, O. (2006) A Study on Reproductive Performance and Related Factors of Zebu Cows in Pastoral Herds in a Semi-Arid Area of Tanzania. *Theriogenology*, **65**, 1859-1874. https://doi.org/10.1016/j.theriogenology.2005.10.016

[34] Kanuya, N.L., Matiko, M.K., Nkya, R., Bittegeko, S.B.P., Mgasa, M.N., Reksen, O., *et al*. (2006) Seasonal Changes in Nutritional Status and Reproductive Performance of Zebu Cows Kept under a Traditional Agro-Pastoral System in Tanzania. *Tropical Animal Health and Production*, **38**, 511-519. https://doi.org/10.1007/s11250-006-4419-z

[35] Ellis, R.W., Rupp, G.P., Chenoweth, P.J., Cundiff, L.V. and Lunstra, D.D. (2005) Fertility of Yearling Beef Bulls during Mating. *Theriogenology*, **64**, 657-678. https://doi.org/10.1016/j.theriogenology.2005.05.029

[36] Chenoweth, P.J. (2000) Rationale for Using Bull Breeding Soundness Evaluation. *Compendium on Continuing Education for the Practising Veterinarian*, **22**, S48-S55.

[37] Wiltbank, J.N. and Parish, N.R. (1986) Pregnancy Rate in Cows and Heifers Bred to Bulls Selected for Semen Quality. *Theriogenology*, **25**, 779-783. https://doi.org/10.1016/0093-691x(86)90093-2

[38] Palasz, A.T., Cates, W.F., Barth, A.D. and Mapletoft, R.J. (1994) The Relationship between Scrotal Circumference and Quantitative Testicular Traits in Yearling Beef Bulls. *Theriogenology*, **42**, 715-726. https://doi.org/10.1016/0093-691x(94)90388-y

[39] Moura, A.A. and Erickson, B.H. (1997) Age-Related Changes in Peripheral Hormone Concentrations and Their Relationships with Testis Size and Number of Sertoli and

Germ Cells in Yearling Beef Bulls. *Reproduction*, **111**, 183-190. https://doi.org/10.1530/jrf.0.1110183

[40] Bourgon, S.L., Diel de Amorim, M., Chenier, T., Sargolzaei, M., Miller, S.P., Martell, J.E., *et al*. (2018) Relationships of Nutritional Plane and Feed Efficiency with Sexual Development and Fertility Related Measures in Young Beef Bulls. *Animal Reproduction Science*, **198**, 99-111. https://doi.org/10.1016/j.anireprosci.2018.09.007

[41] Chacon, J., Perez, E., Muller, E., Soderquist, L. and Rodriguez-Martinez, H. (1999) Breeding Soundness Evaluation of Extensively Managed Bulls in Costa Rica. *Theriogenology*, **52**, 221-231.

[42] Trocóniz, J.F., Beltrán, J., Bastidas, H., Larreal, H. and Bastidas, P. (1991) Testicular Development, Body Weight Changes, Puberty and Semen Traits of Growing Guzerat and Nellore Bulls. *Theriogenology*, **35**, 815-826. https://doi.org/10.1016/0093-691x(91)90422-a

[43] Brito, L.F.C., Silva, A.E.D.F., Barbosa, R.T. and Kastelic, J.P. (2004) Testicular Thermoregulation in Bos Indicus, Crossbred and Bos Taurus Bulls: Relationship with Scrotal, Testicular Vascular Cone and Testicular Morphology, and Effects on Semen Quality and Sperm Production. *Theriogenology*, **61**, 511-528. https://doi.org/10.1016/s0093-691x(03)00231-0

[44] Brito, L.F.C., Silva, A.E.D.F., Unanian, M.M., Dode, M.A.N., Barbosa, R.T. and Kastelic, J.P. (2004) Sexual Development in Early- and Late-Maturing Bos Indicus and Bos Indicus × Bos Taurus Crossbred Bulls in Brazil. *Theriogenology*, **62**, 1198-1217. https://doi.org/10.1016/j.theriogenology.2004.01.006

[45] Kashoma, I.P.B., Luziga, C. and Mgongo, F.O.K. (2010) Bull Selection and Use for Improved Performance in Pastoral Herds of Tanzania. Livestock Research for Rural Development.

[46] Fordyce, G., Howitt, C., Holroyd, R., O'Rourke, P. and Entwistle, K. (1996) The Performance of Brahman-Shorthorn and Sahiwal-Shorthorn Beef Cattle in the Dry Tropics of Northern Queensland. 5: Scrotal Circumference, Temperament, Ectoparasite Resistance, and the Genetics of Growth and Other Traits in Bulls. *Australian Journal of Experimental Agriculture*, **36**, 9-17. https://doi.org/10.1071/ea9960009

[47] Prasanthi, G.S., Patel, R.K., Rao, K.R.S.S. and Singh, K.M. (2004) Detection of Fragile Site on Chromosomes of Sub-Fertile Cattle Bulls. *Intas Polivet*, **5**, 255-261.

[48] Kumaresan, A., Das Gupta, M., Datta, T.K. and Morrell, J.M. (2020) Sperm DNA Integrity and Male Fertility in Farm Animals: A Review. *Frontiers in Veterinary Science*, **7**, Article 321. https://doi.org/10.3389/fvets.2020.00321

[49] Elango, K., Kumaresan, A., Talluri, T.R., Raval, K., Paul, N., Peter, E.S.K.J., *et al*. (2022) Impact of Sperm Protamine on Semen Quality and Fertility. *Journal of Reproductive Healthcare and Medicine*, **3**, Article 5. https://doi.org/10.25259/jrhm_2_2022