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A Descriptive Analysis of Breeding Soundness Evaluation Study in Mpwapwa Breed Bulls Based on Established Criteria for the Use of Semen in Artificial Insemination Programmes

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Abstract

The goal of this study was to conduct a descriptive analysis of a BSE study in Mpwapwa breed bulls at TALIRI Mpwapwa research centre based on established criteria for the use of semen in Artificial Insemination (AI) programmes. In the Tanzanian cattle breeding system, breeding soundness evaluation (BSE) is not considered as a common strategic breeding tool that can be used to evaluate bulls' fertility and improve herd reproductive performance and productivity. Despite of being a synthetic dual purpose breed kept for meat and milk production in the arid and semi-arid areas of Tanzania, the potential of Mpwapwa cattle has never been properly realised. The breed had tremendously contributed to the progress of some of the pastoralists and agro-pastoralists in the Tanzanian beef industry, through upgrading of the Tanzania Short horned Zebu ecotypes via crossbreeding. The results from this study indicate that: in most observations, SC for younger bulls were closely similar to SC for older bulls; milk and cream were the highest and lowest recorded colour; and ++ and + were the highest and lowest recorded mass activity/gross motility; 20% and 90% were the highest and lowest recorded individual motility; 50% and 90% were the highest and lowest recorded morphology; and 966 sperm/mL (x10^6) and 57 sperm/mL (x10^6) were the highest and lowest recorded concentration. Therefore, observed diverse variations particularly in age, SC and semen qualities suggest that Mpwapwa bulls with the best BSE outcomes on SC and semen qualities should be selected for use during the breeding season.
Keywords Mpwapwa breed cattle, Breeding soundness evaluation, Artificial insemination programme and Reproductive performance.

Introduction

The Mpwapwa breed is a synthetic breed, developed from improved Indian dairy breeds (about 60%), African zebu breeds (30%) and European dairy breeds (10%), in order to create a dual-purpose breed, suitable for low to medium production Tanzanian farms. Compared to local Tanzanian zebu cattle, Mpwapwa cattle have higher growth rates and produce four times as much milk, while still having the same level of disease resistance and producing bulls that can be used for draught power (Chawala *et al.*, 2017).

However, the potential of Mpwapwa cattle has never been properly realised. For example, the average milk yield of Mpwapwa cattle is currently no better than it was in the late 1970s (Chawala *et al.*, 2017). The principal driver of this lack of change is that in the 1980s and 90s, structural adjustments to IMF and World Bank policies led to significant reductions in spending in Tanzania on agricultural research as well as privatisation or defunding of parastatal farms, which were crucial to maintaining the development of the Mpwapwa breed. This meant that, genetic development of the Mpwapwa breed was primarily left to semi-commercial smallholder Tanzanian farmers who used natural mating. This reduced selection pressure led to reduced performance of Mpwapwa cattle.

Nonetheless, Mpwapwa breed cattle have contributed to the progress of the Tanzanian beef industry, principally through upgrading of the Tanzania Shorthorn Zebu ecotypes via crossbreeding, with the breed's better performance in terms of growth, disease resilience, and meat characteristics resulting in high demand from pastoralists and agro-pastoralists (Kabuni, 2017).

Thus, increased emphasis on improving the performance of Mpwapwa cattle is likely to have significant benefits for Tanzanian beef production (Kabuni and Laven, 2021). Despite recent changes in policy and government support which mean that selection to improve both productivity and reproductive performance is now feasible (Chawala *et al.*, 2017), significant issues remain, and achieving these selection goals will require a systematic programme of genetic improvement, with cows being mated to the best bulls on both research stations *and* smallholder farms (Chawala *et al.*, 2017). To achieve the latter will require artificial mating and fertility management programmes, both of which are rare on Tanzanian farms.

These programmes will require high quality semen from fertile high genetic merit bulls (Chenoweth *et al.*, 2010). The bulls in the Mpwapwa herd at the Tanzanian Livestock Research Institute (TALIRI) research farm in Mpwapwa are known to have good genetic merit, but we lack good information on their ability to produce high quality semen. The aim of this study was to use a bull breeding soundness evaluation (BSE) to assess the general fertility and semen quality of bulls in the TALIRI Mpwapwa herd, prior to their use in an artificial insemination programme.

Materials and Methods

Study area

TALIRI Mpwapwa is located just outside the town of Mpwapwa in the Dodoma region of Tanzania and is 1000 m above sea level. The two major seasons in the region are dry (May to November) and wet (December to April), with cattle being bred in two breeding seasons: March to May and September to November. The mean daily temperature ranges between 24 and 29°C and annual rainfall is ~720 mm (nearly all of which falls during the rainy season). This study was conducted in February 2021 in the middle of the wet season, prior to the start of the March-May breeding season.

Selection of bulls

All animal-related assessments were approved prior to study start by TALIRI. All bulls that were involved in the study came from the TALIRI Mpwapwa research herd, which in February 2021 had 120 mature (≥24 months) Mpwapwa breed bulls. Bull with a recorded history of previous reproductive problems were excluded (n= 20), and the remaining 100 bulls were then stratified by age (≥24 – ≤28 months, ≥29 – ≤33 months, ≥34 – ≤38 months, ≥39 – ≤43 months, >44 months). Bulls were weighed and body condition scored, and the heaviest 53 bulls were selected for BSE study.

Animal health management and pre-BSE nutrition

After selection, all bulls were weighed, body condition scored, and treated with 5 mg/kg levamisole (Levamizole, Eagle Vet. Tech Co. Kenya). For the five-week period preceding the BSE, bulls were given unrestricted grazing (stocking rate of 0.36 livestock unit/ha) in four 15 ha paddocks. The principal grass species in these paddocks were; *Cenchrus ciliaris, Hyperrhenia rufa, Themeda spp, Cynodon dactylon and*

Chloris gayana. In addition, bulls were trough-fed 0.6 kg/bull/day of a concentrate 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. Details of mineral content of the blocks are in supplementary material. No mineral testing was undertaken at any stage of the study, during this period, bulls were dipped weekly in a bath containing 100 g/L of alphacypermethrin (Paranex, Farm base Ltd, Tanzania). Finally, in March 2021, BCS was assessed and weight measured as the bull left the crush after a one month of deworming, supplementation and grazing management.

Allocation to treatment

The 53 selected bulls were grouped based on number order and ear tag number. They were then divided into five BSE Groups (n=10/11) based on that order, with the first bull going to BSE Group 1, the second bull going to BSE Group 2 and so on.

BSE Procedures

Ten bulls were examined per day over a period of five days. All BSE components/tests were conducted based on the procedures outlined by Kastelic and Thundathil (2008):

a) Examination of structural and reproductive soundness (Physical examination)

Bulls were observed while walking on a hard surface. Legs, joints and muscles were checked for lameness and locomotion problems that could affect mating ability, sight was then checked. For examination of reproductive soundness, bulls were confined in a crush and the scrotum and testes palpated, to ensure that they were symmetrical, non-painful and smooth and that the testes were moving freely within the scrotum, similar checks were undertaken for the epididymis and spermatic cords. The prepuce and penis were then checked for hygiene and the absence of abnormalities.

b) Measurement of scrotal circumference (SC)

The testes were then pushed to the bottom of the scrotum and a standard cloth tape placed around the scrotum at its widest point with moderate tension and scrotal circumference (SC) measured.

c) Collection and examination of semen

Semen was then collected by electro ejaculation (Ejakulator, Minitube GmbH, Germany)**.** One person was responsible for inserting the probe into the bull's rectum, one person for regulating the voltage machine and one person for collecting the semen. During the process, the bull was observed for erection and ejaculation, and electrical stimulation ceased immediately upon ejaculation. Semen samples were collected into vials using an ambient manufactured artificial vagina (AV) cone. Semen samples were taken to the laboratory within 3 to 4 minutes of collection.

For semen examination, the collected semen samples were evaluated on the following components:

i. Volume and colour

On arrival at the laboratory, the volume of the collected semen sample was recorded, and its colour assessed. Firstly, the absence of blood and urine staining was confirmed and colour then recorded, semen samples were then placed into a water bath set at 36°C.

ii. Motility

Mass activity/gross motility was assessed using undiluted semen placed on a warm slide without a coverslip. Aliquots of semen were then diluted (1:50µl) in Optixcell diluent (IMV, L'Aigle, France) and individual progressive motility assessed. Both motility measures were assessed using a phase-contrast microscope (MBL2000 Kruss Optronic GmbH, Germany) at x100 magnification.

iii. Morphology

This was assessed using unstained diluted semen specimens (x100 magnification) using a phase-contrast microscope (MBL2000 Kruss Optronic GmbH, Germany). A drop of diluted semen was placed on a glass slide then placed on a microscope stage for examination.

iv. Concentration

The concentration of sperms was measured using spectrophotometer (Accuread Photometer, Biochrom Ltd, USA). A single drop of sodium chloride was drawn and filled in the cavet using a micropipette of 3990 microliter, followed by addition of one drop of raw semen drawn using a micropipette of 10 microliter. The mixture was then thoroughly shaken and placed into spectrophotometer for concentration reading.

Results

Bull age ranged from 28 to 49 months, except for one bull which was 122 months old (median age was 43 months. Median bodyweight at first examination was 230 kg (range 141 -320 kg), at second examination it was 265 kg (range 185 – 365). Median weight gain during pre-breeding 5-week period was 40 kg (range 15 – 72 kg).

There was a moderate correlation between age and body weight at first and second examination (ρ =0.5 and = 0.52 respectively). Removal of the 122 month old outlier resulted in a regression equation of weight at first examination = 1.99 *age (months) + 147.5. The 95% confidence intervals of the slope was 0.81 to 3.17 and for the intercept they were 100.4 – 194.6 ($r^2 = 0.19$).

All bulls passed the structural soundness and had no detectable abnormalities.

Table 1 Structural and Reproduction Soundness prior to BSE study, Weights and Body Condition Score (BCS) prior and post BSE study during the March-May breeding season 2021

BULL PARTICULARS							
ID	**AGE (months)**	**Structural Soundness**	**Reproduction Examination**	**Body weight at selection (kg)**	**BCS at selection (1-5)**	**Body weight post BSE (kg)**	**BCS post BSE (1-5)**
13117	28	Normal	Normal	230	3	265	4
13119	28	Normal	Normal	183	3	255	4
13122	27	Normal	Normal	170	3	223	4
13128	27	Normal	Normal	225	3	265	4
13139	26	Normal	Normal	163	3	215	4
13174	24	Normal	Normal	190	3	230	4
14566	30	Normal	Normal	205	3	245	4
14574	30	Normal	Normal	230	3	270	4
14590	29	Normal	Normal	185	3	225	4
14718	49	Normal	Normal	190	3	230	4
14722	49	Normal	Normal	239	3	279	4
14739	48	Normal	Normal	264	3	300	4
14747	48	Normal	Normal	314	3	340	4
14750	48	Normal	Normal	268	3	304	4
14762	47	Normal	Normal	242	3	282	4
14785	44	Normal	Normal	285	3	325	4
14791	44	Normal	Normal	265	3	305	4
14802	44	Normal	Normal	246	3	289	4
14804	44	Normal	Normal	238	3	278	4
14817	44	Normal	Normal	276	3	316	4
14835	44	Normal	Normal	200	3	241	4
14836	44	Normal	Normal	244	3	274	4
14841	43	Normal	Normal	238	3	278	4
14842	43	Normal	Normal	220	3	262	4
14843	43	Normal	Normal	235	3	275	4
14852	43	Normal	Normal	273	3	313	4
14858	43	Normal	Normal	240	3	280	4

14865	43	Normal	Normal	220	3	260	4
14869	43	Normal	Normal	252	3	292	4
14887	43	Normal	Normal	195	3	235	4
14889	43	Normal	Normal	232	3	272	4
14891	43	Normal	Normal	215	3	255	4
14901	42	Normal	Normal	230	3	270	4
14903	42	Normal	Normal	203	3	245	4
14911	42	Normal	Normal	222	3	260	4
14922	41	Normal	Normal	205	3	245	4
14924	41	Normal	Normal	217	3	257	4
14952	37	Normal	Normal	230	3	268	4
14965	37	Normal	Normal	215	3	250	4
14966	35	Normal	Normal	185	3	225	4
14967	32	Normal	Normal	187	3	227	4
14975	32	Normal	Normal	190	3	230	4
14976	32	Normal	Normal	240	3	280	4
14977	32	Normal	Normal	250	3	290	4
14980	32	Normal	Normal	204	3	244	4
14983	32	Normal	Normal	208	3	250	4
15946	54	Normal	Normal	220	3	261	4
13161	24	Normal	Normal	212	3	252	4
14755	47	Normal	Normal	141	3	185	4
14770	44	Normal	Normal	235	3	277	4
14916	42	Normal	Normal	230	3	270	4
14925	41	Normal	Normal	320	3	365	4
15195	122	Normal	Normal	285	3	300	4

Table 2 Evaluation of fresh semen samples collected from selected Mpwapwa breed bulls in the BSE study during the March-May breeding season 2021

BULL PARTICULARS			**MACROSCOPIC EVALUATION**			**MICROSCOPIC EVALUATION**		**Concentration Sperm/mL ($x10^6$)**
ID	**AGE (months)**	**SC (cm)**	**Volume (mL)**	**Color**	**Mass activity**	**Individual motility (%)**	**Morphology (% normal)**	
13117	28	27	4	Milk	++	60	60	132
13119	28	26	2.5	Light Milk	+	60	60	94
13122	27	25	2	Light Milk	+	70	70	125
13128	27	29	8	Light Cream	++	75	75	829
13139	26	25	2	Light Milk	+	80	80	86
13174	24	26	7	Light Milk	+	70	70	117
14566	30	29	5.5	Light Milk	+	70	75	110
14574	30	28	5	Milk	++	55	65	192
14590	29	26	3.4	Light Milk	+	50	50	108
14718	49	28	5	Milk	++	70	75	137
14722	49	30	14.9	Milk	+	70	70	116
14739	48	27	7.5	Milk	++	75	70	223
14747	48	29	3.3	Milk	++	85	90	123
14750	48	28	5	Milk	++	80	85	286
14762	47	28	4.5	Milk	+++	85	90	266
14785	44	28	4	Milk	++	70	80	263
14791	44	27	6.4	Milk	++	75	70	325

14802	44	28	13	Milk	+++	90	90	240
14804	44	26	5.5	Cream	+++	85	75	820
14817	44	28	3	Cream	++	80	80	202
14835	44	25	8	Milk	++	80	80	205
14836	44	28	4.3	Light Milk	+	75	75	102
14841	43	33	6	Milk	++	80	80	746
14842	43	29	8	Milk	+++	85	80	734
14843	43	25	5	Milk	++	80	80	107
14852	43	28	3	Milk	+++	85	87	227
14858	43	27	4	Milk	++	75	75	403
14865	43	28	5	Milk	++	75	85	137
14869	43	27	5.5	Light Milk	++	75	75	103
14887	43	27	7	Light Milk	+	70	80	57
14889	43	27	7.2	Milk	++	75	80	244
14891	43	28	6.4	Milk	++	85	80	253
14901	42	25	3	Cream	+++	90	80	873
14903	42	27	1.9	Milk	++	70	75	100
14911	42	29	8	Milk	+++	90	80	815
14922	41	26	3.2	Milk	++	80	75	310
14924	41	27	3	Milk	++	70	70	305
14952	37	28	5	Milk	+++	80	80	208
14965	37	33	6.5	Milk	+++	90	90	190
14966	35	28	6	Milk	+	20	70	408
14967	32	28	5.8	Milk	+++	75	80	966
14975	32	26	4.1	Milk	++	70	70	326
14976	32	30	8.2	Milk	++	75	75	201
14977	32	26	3.5	Milk	++	70	70	284
14980	32	26	5.5	Milk	+	70	75	315
14983	32	29	2.8	Cream	+++	80	85	896
15946	54	26	10.5	Milk	+	65	80	111
Bulls from which no sperm-rich fraction was collected								
13161	24	26	2.5	Watery				0
14755	47	27	5	Watery				0
14770	44	27	6	Watery				0
14916	42	29	5	Watery				0
14925	41	24	8	Watery				0
15195	122	32	8	Watery				0
Key to mass motility scores				(0) Poor, no waves and the spermatozoa are immobile		(++) Good, less dark waves with moderate movement		
				(+) Normal, clear waves with very slight movement		(+++) Very good, lots of dark waves moving rapidly		

Semen was successfully collected from 44/50 bulls: only seminal plasma was collected from the remaining 6 bulls.

Discussion

In the Tanzanian cattle breeding system, breeding soundness evaluation (BSE) is not considered as a common strategic breeding tool that can be used to evaluate bulls' fertility and improve herd reproductive performance and productivity. So, the goal of this study was to conduct a descriptive analysis of a BSE study in Mpwapwa breed bulls at TALIRI Mpwapwa research centre based on established criteria for the use of semen in Artificial Insemination (AI) programmes. A general physical examination was conducted with more attention paid to structural (i.e. legs, joints, muscles and sight), and reproduction (i.e. scrotum, testes, prepuce, penis and sex glands) soundness. There were no evidence of any condition indicating whether bulls had either fertility or structural problems on their reproductive tract and structural soundness. This was contrary to semen examination where some of the evidences concerning volume, colour, motility, morphology and concentration in some of the bulls were observed to be out of the established standards for the use of semen in AI programmes. All bulls' initial body weight had a BCS of 3 which later after a 4 weeks supplementation period improved to BCS of 4 due to increase in their body weight.

Interestingly, bulls' age and SC were inversely proportional to the majority of the bulls. As such, in most observations, SC for younger bulls were closely similar to SC for older bulls (Table 3). As recommended by McGowan *et at.*, (2002), the minimum SC for a 2 year - old *Bos indicus* and its derived crosses in a herd kept under natural grazing system in the aridity areas, should be ≥ 28cm, with less bulls (about < 3%) in a herd failing to meet this recommendation. He also reported that once the bulls attained puberty, SC continues to increase until it reaches the target SC of 34 cm for the 3+ years old bulls. These findings were also reported by Parkinson (2004) and Silva *et al.*, (2014). However, in this study, the youngest bull had 24 months (2 years) with a SC of 26 cm which is 2 cm behind the recommendation, and the oldest bull had 122 months (10.2 years) with a SC of 32 cm which is 2cm behind the targeted SC (34 cm) for the 3+ years old bulls. Among the 53 bulls involved in the study, bulls that were between 2 and 10.2 years old had different SC irrespective to their ages. Since SC is highly heritable, selecting young (i.e. yearling) Mpwapwa breed bulls with the best SC will result into decreased age at attainance of puberty, and improved fertility and reproductive performances (Parkinson 2004; Quirino and Bergmann, 1998).

Table 3. Trend in the relationship between age and SC in some of the older and younger Mpwapwa bulls

Bull ID	AGE (months)	SC (cm)
15946	54	26
14718	49	28
14739	48	27
14762	47	28
14835	44	25
14590	29	26
13119	28	26
13122	27	25
13139	26	25
13174	24	26

However, in some of the older bulls, larger SC were observed (Table 4), but again, the relationship between age and SC in these bulls was not linear.

Table 4. Trend in the relationship between age and SC in older Mpwapwa bulls

Bull ID	AGE (months)	SC (cm)
15195	122	32
14722	49	30
14841	43	33
14965	37	33
14976	32	30
14566	30	29

On the other hand, large variations were observed in the parameters (i.e. volume, colour and concentration) examined for the macroscopic evaluation of the collected semen samples. For example: 14.9 and 1.9 were the highest and lowest recorded volume; milk and cream were the highest and lowest recorded colour; and ++ and + were the highest and lowest recorded mass activity/gross motility. In contrast, slightly variations were observed for the microscopic evaluation particularly in the individual motility and morphology except for the concentration where large variations were noticed. For example: 20% and 90% were the highest and lowest recorded individual motility; 60% and 90% were the highest and lowest recorded morphology; and 966 sperm/mL (x10^6) and 57 sperm/mL (x10^6) were the highest and lowest recorded concentration. Regardless of producing reasonable volume, the semen collected from eight bulls were recorded to have watery colouration with no sperm-rich fraction. In addition, some of the older and younger bulls with the highest SC managed to produce semen of acceptable volume (4 - 8 mL), with acceptable percentage of sperm with good motility (40 - 75 %), morphology (65 – 90 %), and concentration (800 – 1500 million/mL), with minimal variations across volume, motility, morphology and diverse variations on concentration (Table 5).

Table 5. Trend in the relationship between age, SC, volume, motility, morphology and concentration for semen samples collected from some older and younger Mpwapwa bulls

Bull ID	Age (months)	SC (cm)	Volume (mL)	Motility		Morphology (%)	Concentration Sperm/mL (x10^6)
				Mass activity/Gross motility	Individual motility (%)		
14722	49	30	14.9	+	70	70	116
14841	43	33	6	++	80	80	746
14965	37	33	6.5	+++	90	90	190
14976	32	30	8.2	++	75	75	201
14566	30	29	5.5	+	70	75	110
13174	24	26	7	+	70	70	117

Barth (2000) and Menegassi *et al.*, (2011), reported that larger testicles (SC) are usually observed in bulls from large maturing breeds compared to bulls from small maturing breeds. They pointed out that bulls from the *Bos taurus* breeds (i.e. large maturing breeds) normally have larger testicles (SC) compared to the bulls from the *Bos indicus* breeds. Based on these grounds, there is a linear relationship existing between breed, age, SC and the characteristics of the produced semen. Thus, regardless of the breed, as the age of the bull increases after puberty, the volume and quality of the produced semen also increase as well. However, this relationship ceases once the bull is too old.

Conclusion

Therefore, one of the conclusions that can be drawn on the BSE outcomes of Mpwapwa bulls is that; the observed diverse variations particularly on age, SC and semen qualities suggest that Mpwapwa bulls with the best BSE outcomes on SC and semen qualities should be selected for use during the breeding season. This is because the use of such bulls in a well organised breeding programme can result into production of improved progeny with improved performance, consequently this can lead to improved herd's reproductive performances and productivity. Bulls with poor BSE outcomes on SC and semen qualities on the other hand should be culled from the breeding bulls because their use can result into reduced reproductive performances and productivity.

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Conflict of Interest

Authors do not have any conflict of interest.

Authors Contribution

Authors contributed in the designing, coordination, supervision and implementation of the study. They were also involved in the development of the manuscript.

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