African Journal of Biotechnology Vol. 10(64), pp. 14223-14229, 19 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2010
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Comparative study on semen characteristics of Kolbroek and Large White boars following computer aided sperm analysis® (CASA)

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Accepted 9 September, 2011

Consistent estimates of boar fertility potential from objective semen evaluation could be a valuable tool for boar selection. The objective of this study was to evaluate semen characteristics of Kolbroek and Large White boars following computer aided sperm analysis® (CASA). Eight ejaculates were collected separately from individual Kolbroek (n = 4) and Large White (n = 4) boars using the gloved-hand technique. Following semen collection, semen was evaluated for macroscopic and microscopic characteristics. Analysis of variance (ANOVA) was used to test the differences between the breeds (P<0.05). The bodyweight of Kolbroek (154.7 ± 8.5) was significantly lower compared to Large White (189.9 ± 7.7) boar. There was also a positive correlation between bodyweight and semen volume of both Kolbroek (r = 0.2197) and Large White (r = 0.2577) boar. However, no significant differences were observed in Kolbroek and Large White boar semen volume (140 and 170 ml), sperm concentration (0.727 and 0.761 × 10^9 sperm cell/ml), pH (7.0 and 7.0), total motility (95 and 91%) and morphology (84 and 82%). In conclusion, the bodyweight of Kolbroek and Large White boar was positively correlated with ejaculated semen volume. Sperm characteristics of both Kolbroek and Large White boar were similar. Sperm class analyser® provided a precise and more objective information of sperm motility characteristics.

Key words: Sperm, Large White, Kolbroek, motility rate, boar.

INTRODUCTION

Large White is the most popular exotic breed in South Africa (ARC, 1993) due to their superior fertility and growth rate (Ncube et al., 2003). However, their high nutrient requirements and intensive management systems make them unsuitable for resource-poor rural farmers and harsh environmental conditions. Kolbroek is a South African indigenous pig breed with unique genetic traits for diseases tolerance and adaptability in harsh environmental conditions (Ramsay et al., 1994). They are considered appropriate breed for the resource-poor rural farmers because of their tolerance to various diseases and capacity to utilize fibrous and poor quality feed resources compared to exotic breeds (Halimani et al., 2010).

A recent survey indicated a catastrophic collapse in the population of South African indigenous germplasm (FAO, 2007). This collapse was attributed among others to unplanned breeding, crossbreeding and introduction of exotic germplasm (Scholtz, 2005). Mating and cross-breeding are largely unsupervised leaving these breeds vulnerable to inbreeding and uncontrolled genetic

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admixture with other breeds (Halimani et al., 2010). Most researches have been focused on the imported genotypes which cannot be sustained under smallholder conditions (Ncube et al., 2003). Hence, there is a need to evaluate reproductive potential of imported boars in comparison with South African indigenous boars. The reproductive potential of the indigenous Kolbroek boars has not been fully exploited in South Africa compared to other pig genotypes. A proper semen analysis is empirical for boar selection in the herd and for preserving their genetic materials through *ex-situ* and *in-situ.* Indigenous pigs were long regarded as unsuitable for intensive commercial breeding because of their slow growth and inadequate meat production (Prolit, 2004). However, indigenous pigs exhibit well-established adaptations to severe environmental and management conditions (Swart et al., 2010). Moreover, there is lack of accurate method of predicting the fertility rate of Kolbroek boar sperm to determine their reproductive potential.

Sperm motility is known to be an important characteristic in predicting the fertility of male potential performances (Holt et al., 1997; Tardif et al., 1999; Gadea, 2005). However, subjective microscope evaluation varied between 30 to 60% from the same ejaculates (Amann, 1989). Due to these biases, emphasis has been placed on the use of objective methods such as Computer Aided Sperm Analysis® (CASA) system (Saikhun et al., 2011). Therefore, the objective of this study was to compare South African indigenous Kolbroek and exotic Large White boar breeds on sperm characteristics following analysis by computer aided sperm Analysis® (CASA) known as Sperm Class Analyser® (SCA).

MATERIALS AND METHODS

The study was conducted at the Pig Research Unit of Agricultural Research Council (Germplasm Conservation & Reproductive Biotechnologies Unit), Irene, South Africa. The Agricultural Research Council-Irene campus is located at 25° 55' South; 28° 12' East. The institute is located in the Highveld region of South Africa and situated at an altitude of 1525 m above sea level. Four indigenous Kolbroek and four exotic Large White boars were used for this study because of the scarcity challenge of finding Kolbroek boars. The boars were between 2 to 3 years of age. The study was done in summer season (February to March, 2011). The boars were weighed using a KM3 electronic weight indicator (Rudoweigh®). The boars were in good health condition throughout the duration of the study. The diets were formulated to meet the nutritional requirements of the boars (crude protein (CP): 13% and digestible energy (DE): 13 MJ/kg) (National Research Council, 1998). Water was given *ad libitum* throughout the duration of the study.

Semen collection and processing

Semen samples were collected from the experimental boars twice weekly from February to March. Twelve Ejaculates were collected separately from four Kolbroek and four Large White boars with the gloved-hand technique in a 300-ml glass beaker. The filtered semen fraction were sealed with a gauze filter inside a pre-warmed (39°C) insulated thermos flask. Upon arrival at the laboratory, semen volume was measured by using the graduated falcon tube, pH was measured using the litmus paper, then sperm concentration was measured using the spectrophotometer (Jenway 6310 spectrophotometer, Bibby Scientific, England) and was recorded in billions ($\times 10^9$/ml). Experimental boars were cared for according to the guidelines for the Agricultural Research Council, Animal Production Institute ethics committee (Ref: APIEC10/01).

Sperm morphology

Semen was collected from Kolbroek and Large White boars and a 10× dilution was prepared by adding semen to 0.9% sodium chloride. One drop of 0.27% Chicago sky blue and one drop of diluted semen were mixed on a slide. Slides were air-dried in a near vertical position then put into a fixative in a jar for 2 min and then rinsed with tap and distilled water. Slides were put into jars containing the Giemsa staining solution and left for 20 h at room temperature. The slides were rinsed again in tap and distilled water for 2 min, air-dried in a near vertical position and cover slipped with methyl yellow. A drop of oil immersion (Olympus, Japan) was placed on the smeared microscope glass slide and 100 sperm were counted at 100 × magnification (Figure 1A and B). A criterion was applied for the evaluation for abnormal sperm head (flat, sharp, double and if it is not oval); midpiece (proximal and distal cytoplasmic droplets); tail (coiled, double, broken). A live sperm was white/pink in colour and a dead sperm was dark blue (Kovács and Foote, 1992).

Sperm motility rate

The 10 µL of raw semen were placed into 500 µL of BO wash medium in 15 ml tube (Falcon® 352099, USA). The tube was then kept in CO_2 incubator (Sanyo, Japan) adjusted to 39°C. Five micro litres of semen was placed on the warm glass slide (~76 × 26 × 1 mm, Germany) and placed with a warmed cover slip (22 × 22 mm, Germany) over the microscope-warm plate (Omron) adjusted at 39°C. The sperm motility rates were evaluated by computer assisted sperm analysis system (Sperm Class Analyzer® [SCA] 5.0, Microptic, Barcelona, Spain) at the magnification of 10 × (Nikon, China). The kinematic values recorded for each sperm included, in addition to the overall percentage of motile sperm, the velocity of movement, the width of the sperm head's trajectory and the frequency of the change in direction of the sperm head (Table 1).

Data analysis

The analysis was done using Genstat Software. The experiment was designed as a completely randomised design with two treatments (Kolbroek and Large White boars). Analysis of variance (ANOVA) was used to test for differences between the treatments. The data were acceptably normal with homogeneous treatment variances. Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at a significant level of $P<0.05$ (Snedecor and Cochran, 1980). The correlation of the bodyweight with semen volume, concentration and sperm motility was performed using SAS statistical software. The Pearson two-sided was used to determine the correlation between bodyweight and the variables (Snedecor and Cochran, 1980).

RESULTS

The results of macroscopic evaluations are outlined in

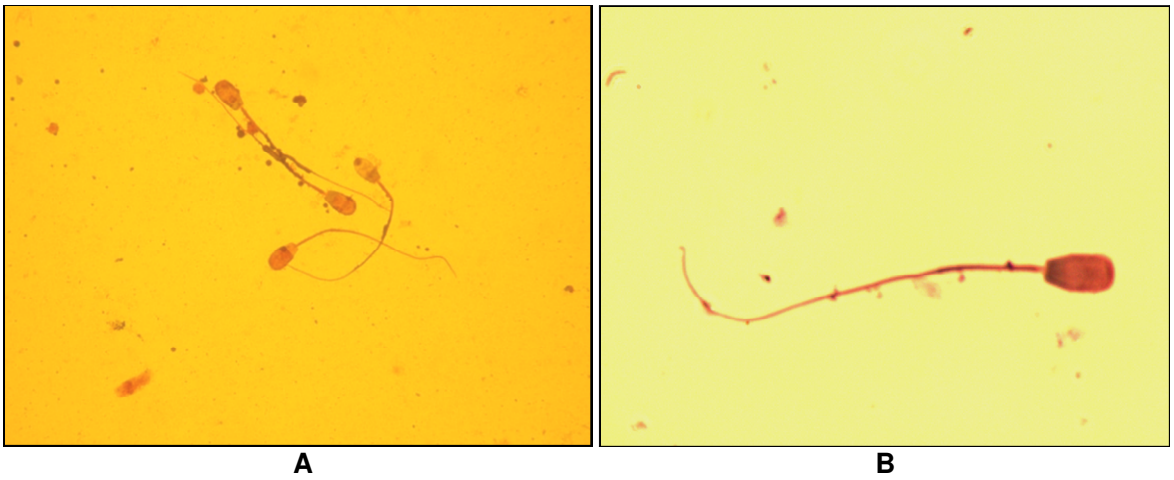

Figure 1. (A): Kolbroek live sperm; **(B)** Kolbroek dead sperm.

Table 1. Sperm class analyzer® settings used to analyse sperm motility and velocity parameters.

Parameter	Setting
Contrast	169
Brightness	470
Image/second	50
Optic	Ph-
Chamber	Cover slide
Scale	10X
Particle size (μm^2)	10<70
Slow (µm/s)	<40
Medium (µm/s)	<80
Rapid (µm/s)	<120
Progressivity (%)	40% of straightness
Circular (%)	50% of linearity
Connectivity	11
Velocity on the average path points	7
Number of images	50

Table 2. Macroscopic evaluation for Kolbroek and Large White boar semen.

Breed	Bodyweight (kg)	Semen volume (ml)	Semen pH	Semen concentration (× 10^9 sperm cell/ml)
Kolbroek 1	166.5	130.0 ± 26.5	7.0 ± 0.0	0.533.4 ± 90.8
Kolbroek 2	150.5	126.7 ± 11.6	7.0 ± 0.0	1.0521 ± 283.3
Kolbroek 3	147.0	100.0 ± 0.0	7.0 ± 0.0	0.9073 ± 333.1
Kolbroek 4	155.0	205.0 ± 37.8	7.0 ± 0.0	0.4153 ± 174.2
Averages	154.7 ± 8.5[a]	140.4 ± 48.6	7.0	0.727 ± 340.8
Large White 1	196.6	226.7 ± 100.2	7.0 ± 0.0	0.646.7 ± 82.2
Large White 2	190.6	145.0 ± 42.7	7.0 ± 0.0	0.605.0 ± 328.0
Large White 3	179.0	180.0 ± 45.8	7.0 ± 0.0	0.590.3 ± 135.9
Large White 4	193.4	158.3 ± 18.9	7.0 ± 0.0	1.203.4 ± 487.9
Averages	189.9 ± 7.7[b]	177.5 ± 60.4	7.0	0.761. 0 ± 372.8

[ab]Different letters indicate significant differences within columns (P<0.05).

Table 3. Sperm morphology and viability for Kolbroek and Large White boar semen (±SD).

Breed	Live (%)	Dead (%)	Abnormality (%)		
			Head	Midpiece	Tail
Kolbroek 1	88.7 ± 3.1	9.7 ± 4.0	0.7 ± 1.2	0.0 ± 0.0	1.0 ± 1.7
Kolbroek 2	84.7 ± 7.5	10.0 ± 8.7	2.3 ± 0.6	1.7 ± 2.9	1.3 ± 1.2
Kolbroek 3	82.0 ± 1.0	13.3 ± 6.0	2.7 ± 3.8	1.0 ± 1.7	1.0 ± 1.7
Kolbroek 4	83.0 ± 10.0	9.7 ± 5.5	2.3 ± 2.3	1.0 ± 1.7	4.0 ± 3.5
Averages	84.6 ± 6.1	10.7 ± 5.6	2.0 ± 2.1	0.9 ± 1.7	1.8 ± 2.3
Large White 1	82.0 ± 9.6	7.0 ± 5.3	0.7 ± 0.6	0.3 ± 0.6	6.3 ± 6.0
Large White 2	87.7 ± 6.7	5.3 ± 1.5	2.7 ± 1.5	1.0 ± 1.0	6.0 ± 6.0
Large White 3	80.7 ± 5.5	9.3 ± 5.0	1.7 ± 2.1	0.0 ± 0.0	5.7 ± 7.4
Large White 4	76.3 ± 3.5	17.3 ± 7.0	0.7 ± 0.6	1.3 ± 1.2	8.0 ± 4.4
Averages	81.7 ± 7.1	9.8 ± 6.3	1.4 ± 1.4	0.7 ± 0.9	6.5 ± 5.2

Table 2. The bodyweight of Kolbroek (154.7 ± 8.5 kg) was significantly lower compared to Large White (189.9 ± 7.7 kg) boar. There was a positive correlation between bodyweight and semen volume of Kolbroek (r = 0.2197) and Large White (r = 0.2577). Conversely, there was a negative correlation between bodyweight and sperm motility rate (r = -0.9655) and concentration (r = -0.6600) of Kolbroek. However, the bodyweight of Large White was positively correlated with sperm concentration (r = 0.3721), but negatively correlated to total motility (r =-0.1043). No significant differences were observed in Kolbroek and Large White boar volume (140 and 170 ml), semen pH (7.0 and 7.0) and sperm concentration (0.727 and 0.761 × 10^9 sperm cell/ml). Furthermore, no individual variation was observed for semen volume, pH and concentration.

The results for Kolbroek and Large White sperm morphology are presented in Table 3. The average percentage (±SD) of Kolbroek and Large White live sperm was 84.6 ± 6.1 and 81.7 ± 7.1%, respectively. There was no significant differences ($P<0.05$) in abnormal sperm morphology of Kolbroek and Large White. More also, the results of both Kolbroek and Large White sperm motility are presented in Table 4. The average percentage (±SD) of Kolbroek and Large White sperm motility was 95.2 ± 4.2 and 91.4 ± 6.2%, respectively. However, a significant difference was observed for rapid sperm motility of Kolbroek 4 (79.4 ± 2.6%) as compared to all the other boars including Large White. No significant difference was observed for all other sperm motility and velocity parameters for Kolbroek and Large White boar.

DISCUSSION

This study demonstrates that the bodyweight of Kolbroek (154.7 ± 8.5) was significantly lower compared to Large White (189.9 ± 7.7) boar. There was also a bodyweight correlation to semen volume ejaculated by both Kolbroek (r = 0.2197) and Large White (r = 0.2577). However, no significant differences were observed for Kolbroek and Large White boar semen volume (140 and 170 ml), sperm concentration (0.727 and 0.761 × 10^9 sperm cell/ml), pH (7.0 and 7.0), total motility rate (95 and 91%) and morphology (84 and 82%). Similarly, it was previously reported that breed did not have a significant effect on boar sperm characteristics (Kennedy and Wilkins, 1984; Rothschild, 1996; Oh et al., 2003). Kolbroek boars had a slightly lower semen volume as compared to the standard semen volume of 150 to 300 ml in exotic breeds (Kondracki, 2003). Egerszegi et al. (2008) reported similar results for Hungarian indigenous Mangalica boars (178 ml). In contrast, Wolf and Smithal (2009) found that Czech Large White and Landrace had a slightly higher semen volume of 276 and 273 ml, respectively.

Furthermore, Chimonyo et al. (2005) reported that indigenous pigs in southern Africa are smaller in size compared to exotic pig breeds. This was evident in the present study as Kolbroek boars had a lower bodyweight (154.8 kg) compared to Large White boar (189.9 kg). Larger breeds such as Large White tend to produce higher semen volume (Hughes and Varely, 1980). Similarly, same results were observed in the present study as semen volume of both boar breeds was influenced by bodyweight. Although, Kolbroek boar bodyweight was lower, the sperm concentration was higher. There was a negative correlation between bodyweight and sperm motility rate (r = -0.9655) and concentration (r = -0.6600); but positively correlated with volume (r = 0.2197) of Kolbroek boar. However, the bodyweight of Large White was positively correlated with volume (r = 0.2577) and sperm concentration (r = 0.3721), but negatively correlated to total motility (r = -0.1043).

Moreover, Johnson et al. (2000) reported that the pH of raw boar semen varies between 7.0 and 7.5, irrespective of the boar breed. This is in agreement with the present

Table 4. Sperm motility and velocity rates for Kolbroek and Large White boars (±SD).

Boar	Sperm motility			Sperm velocity					
	TM (%)	RAP (%)	PM (%)	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)	LIN (%)	STR (%)	WOB (%)
Kolbroek 1	91.8 ± 6.6[a]	35.5 ± 1.2[b]	36.3 ± 25.8[a]	135.2 ± 31.1[a]	42.8 ± 21.2[a]	82.7 ± 12.4[a]	33.8 ± 20.8[a]	51.4 ± 21.8[a]	64.4 ± 21.2[a]
Kolbroek 2	96.2 ± 3.4[a]	54.0 ± 8.0[b]	31.1 ± 4.6[a]	133.6 ± 12.1[a]	39.4 ± 5.8[a]	97.7 ± 18.9[a]	29.6 ± 4.7[a]	40.8 ± 5.1[a]	72.9 ± 9.3[a]
Kolbroek 3	96.6 ± 2.6[a]	52.1 ± 9.9[b]	31.3 ± 9.9[a]	132.7 ± 1.9[a]	35.5 ± 3.0[a]	84.0 ± 20.9[a]	26.7 ± 2.0[a]	43.3 ± 6.4[a]	63.2 ± 14.8[a]
Kolbroek 4	96.1 ± 3.5[a]	79.4 ± 2.6[a]	48.7 ± 13.3[a]	171.7 ± 11.2[a]	46.0 ± 5.4[a]	98.0 ± 12.9[a]	26.8 ± 2.2[a]	47.7 ± 9.3[a]	57.1 ± 7.0[a]
Averages	95.2 ± 4.2	55.2 ± 17.3	36.8 ± 15.2	143.3 ± 22.8	40.9 ± 10.6	90.6 ± 16.1	29.2 ± 9.7	45.8 ± 11.5	64.4 ± 13.5
Large White 1	94.8 ± 5.2[a]	46.4 ± 21.2[b]	27.9 ± 8.2[a]	136.2 ± 37.2[a]	36.0 ± 9.8[a]	86.8 ± 27.3[a]	26.8 ± 5.4[a]	42.3 ± 9.0[a]	63.3 ± 2.5[a]
Large White 2	87.2 ± 10.6[a]	39.2 ± 1.0[b]	15.3 ± 3.6[a]	121.2 ± 11.3[a]	27.7 ± 1.0[a]	77.7 ± 2.6[a]	23.0 ± 3.1[a]	35.7 ± 2.5[a]	64.4 ± 4.1[a]
Large White 3	93.5 ± 2.9[a]	38.3 ± 11.8[b]	24.4 ± 2.6[a]	121.2 ± 16.0[a]	33.8 ± 5.6[a]	73.3 ± 10.5[a]	28.6 ± 8.8[a]	47.6 ± 15.6[a]	60.4 ± 2.1[a]
Large White 4	89.9 ± 3.7[a]	44.0 ± 18.1[b]	23.6 ± 6.6[a]	137.7 ± 33.3[a]	33.8 ± 8.9[a]	86.7 ± 25.7[a]	24.5 ± 1.2[a]	39.4 ± 1.7[a]	62.3 ± 5.1[a]
Averages	91.4 ± 6.2	42.0 ± 13.4	22.8 ± 6.8	129.1 ± 24.8	32.8 ± 7.0	81.1 ± 17.7	25.7 ± 5.1	41.2 ± 9.0	62.6 ± 34

TM, Total motility; RAP, Rapid; PM, progressive motility; VCL, velocity on the curve line; VSL, velocity on the straight line; VAP, velocity on the average path; LIN, linearity; STR, straightness; WOB, wobble. [ab]Different letters indicate significant differences ($P<0.05$).

observed pH results (7.0) in both breeds. However, a pH change (increase or decrease) is detrimental to both the sperm metabolism and motility. Infection is usually associated with alkaline ejaculate (pH>8.0), which leads to diminished sperm motility and an increased proportion of altered acrosomes (Althouse et al., 2000). In the present study, the boar semen pH did not negatively affect the sperm motility. In addition, no differences were observed for Kolbroek and Large White boar sperm concentration. Variation in the number of sperm in an ejaculate has been described bet-ween different pig breeds (Kommisrud et al., 2002), which is a first factor influencing semen dose production. Not only differences in sperm concentration but also in sperm volume (Kondracki, 2003), influence sperm concentration. The sperm concentration for indigenous Kolbroek was higher (0.727×10^9 sperm cell/ml) as compared to the Hungarian Mangalica boar (0.490×10^9 sperm cell/ml) (Egerszegi et al., 2008).

The percentage of sperm with normal morphology was above 80% for Kolbroek and Large White boars. Such percentages of normal morphology are correlated with fertility (Sanchez et al., 1998; Xu et al., 1998; Alm et al., 2006). The results from this study also showed that there are no variations between individual boars, irrespective of the breed. Similar findings were observed by Borg et al. (1993) who reported that characteristics of sperm morphology did not differ among different boar breeds (Duroc, Meishan, Fengjing and Minzhu boars). Kolbroek and Large White semen showed a lower percentage of morphologically abnormal sperm (4.7 ± 2.0 and 2.9 ± 2.5%, respectively) as compared to other studies. Wolf and Smithal (2009) found a slightly higher percentage of abnormal sperm (11.4 and 11.2%) for Czech Large White and Czech Landrace boars, respectively. Criteria for the maximum percentage of primary and secondary abnormalities in commercial pig AI-centres were determined as 10 and 20%, respectively (Waberski et al., 1994; Flowers, 1997). Morphological abnormalities give an indication of aberrations in the spermatogenesis. Morphological abnormalities of sperm can also have a detrimental impact upon fertilization and embryonic development (Walters et al., 2005; Saacke, 2008).

The average sperm total motility obtained for Kolbroek and Large White was 95 and 91%, respectively. These sperm motility results are an indication of an active metabolism and are considered to be of great importance for fertilization to take place. Lower motility percentages were reported (70.2 ± 8.8%) for Czech hybrid AI boars (Frydrychová et al., 2010). Subjective method was used to evaluate sperm motility analysis. Microscopic techniques have limitations including subjectivity, variability, the small number of sperm analysed and poor correlation with fertilizing potential (Rijsselaere et al., 2005). Subjective visual evaluation of motility is also prone to human error and biasness. Hence, the computer-assisted

sperm analysis (CASA) was initiated to reduce subjective bias on the motility assessment and to discriminate a series of motility patterns of boar semen (Tretipskul et al., 2010).

Conclusion

The bodyweight of Kolbroek and Large White boar was positively correlated with ejaculated semen volume. However, macroscopic and microscopic sperm characteristics of Kolbroek were similar compared to Large White boar. Surprisingly, Kolbroek boar sperm concentration and motility rate was negatively correlated to bodyweight compared to only Large White sperm concentration. This is the first study that provided more information on sperm motility characteristics of both Kolbroek and Large White boar using Sperm Class Analyser®. It is recommended that further studies should be conducted with more number of boars to validate the sperm motility characteristics information following artificial insemination.

ACKNOWLEDGEMENTS

The authors wish to acknowledge National Research Foundation, Agricultural Research Council (ARC) and Department of Agriculture, Forestry and Fisheries (DAFF) for funding the project. Special thanks to Prof. T. L. Nedambale and ARC-Germplasm Conservation and Reproductive Biotechnologies personnel for their assistance.

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