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

Matshidiso Bailekae Masenya¹,², M. L. Mphaphathi¹,³, M. H. Mapeka¹,³, P. H. Munyai¹,⁴, M. B. Makhafofa¹,², F. V. Ramukhithi¹,³, P. P. Malusi¹, D. O. Umesiobi² and T. L. Nedambale¹,³,⁴*

¹Agricultural Research Council, Animal Production Institute, Germplasm Conservation & Reproductive Biotechnologies, Private Bag X2, Irene, 0062, South Africa.
²Department of Agriculture, Central University of Technology, Private Bag X20539, Bloemfontein, 9300, South Africa.
³Tshwane University of Technology, Department of Animal Sciences, Private Bag X680, Pretoria 0001, South Africa.
⁴University of the Free State, Department of Animal, Wildlife and Grassland Sciences, Private Bag X 339, Bloemfontein, South Africa.

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 × 10⁹ 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
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 (Proli, 2004). However, indigenous pigs exhibit well-established admixture with other breeds (Halimani et al., 2010). Most researches have been focused on the imported genotypes.

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 CO₂ 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     | Ph-     |
| Scale                            | 10X     |
| Particle size (µm²)              | 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 (x 10⁹ sperm cell/ml) |
|-------------|-----------------|-------------------|----------|------------------------------------------|
| Kolbroek 1  | 166.5           | 130.0 ± 26.5      | 7.0 ± 0.0| 0.533 ± 90.8                             |
| Kolbroek 2  | 150.5           | 126.7 ± 11.6      | 7.0 ± 0.0| 1.052 ± 283.3                            |
| Kolbroek 3  | 147.0           | 100.0 ± 0.0       | 7.0 ± 0.0| 0.907 ± 333.1                            |
| Kolbroek 4  | 155.0           | 205.0 ± 37.8      | 7.0 ± 0.0| 0.415 ± 174.2                            |
| Averages    | 154.7 ± 8.5     | 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 ± 82.2                             |
| Large White 2| 190.6           | 145.0 ± 42.7      | 7.0 ± 0.0| 0.605 ± 328.0                            |
| Large White 3| 179.0           | 180.0 ± 45.8      | 7.0 ± 0.0| 0.590 ± 135.9                            |
| Large White 4| 193.4           | 158.3 ± 18.9      | 7.0 ± 0.0| 1.203 ± 487.9                            |
| Averages    | 189.9 ± 7.7     | 177.5 ± 60.4      | 7.0      | 0.761 ± 372.8                            |

abDifferent 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 no significant difference in semen volume \((140 \text{ and } 170 \text{ ml})\), sperm concentration \((0.727 \text{ and } 0.761 \times 10^9 \text{ sperm cell/ml})\), pH \((7.0 \text{ and } 7.0)\), total motility rate \((95 \text{ and } 91\%)\) and morphology \((84 \text{ and } 82\%)\). 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 \((178 \text{ ml})\). 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 \((154.8 \text{ kg})\) compared to Large White boar \((189.9 \text{ kg})\). Larger breeds such as Large White tend to produce higher semen volume \((Hughes and Varley, 1980)\). 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 \((r = -0.1043)\).

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 \((140 \text{ and } 170 \text{ ml})\), sperm concentration \((0.727 \text{ and } 0.761 \times 10^9 \text{ sperm cell/ml})\), pH \((7.0 \text{ and } 7.0)\), total motility rate \((95 \text{ and } 91\%)\) and morphology \((84 \text{ and } 82\%)\). 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).
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 ± 6.6a   | 35.5 ± 1.2b   | 36.3 ± 25.8a | 135.2 ± 31.1a | 42.8 ± 21.2a | 82.7 ± 20.8a | 51.4 ± 21.8a | 64.4 ± 21.2a |
| Kolbroek 2 | 96.2 ± 3.4a   | 54.0 ± 8.0b   | 31.1 ± 4.6a  | 133.6 ± 12.1a | 39.4 ± 5.8a  | 97.7 ± 18.9a | 29.6 ± 4.7a  | 40.8 ± 5.1a  | 72.9 ± 9.3a  |
| Kolbroek 3 | 96.6 ± 2.6a   | 52.1 ± 9.9b   | 31.3 ± 9.9a  | 132.7 ± 1.9a  | 35.5 ± 3.0a  | 84.0 ± 20.9a | 26.7 ± 2.0a  | 43.3 ± 6.4a  | 63.2 ± 14.8a |
| Kolbroek 4 | 96.1 ± 3.5a   | 79.4 ± 2.6a   | 48.7 ± 13.3a | 171.7 ± 11.2a | 46.0 ± 5.4a  | 98.0 ± 12.9a | 26.8 ± 2.2a  | 47.7 ± 9.3a  | 57.1 ± 7.0a  |
| 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 ± 5.2a | 46.4 ± 21.2b | 27.9 ± 8.2a | 136.2 ± 37.2a | 36.0 ± 9.8a | 86.8 ± 27.3a | 26.8 ± 5.4a | 42.3 ± 9.0a | 63.3 ± 2.5a |
| Large White 2 | 87.2 ± 10.6a | 39.2 ± 1.0b | 15.3 ± 3.6a | 121.2 ± 11.3a | 27.7 ± 1.0a | 77.7 ± 2.6a | 23.0 ± 3.1a | 35.7 ± 2.5a | 64.4 ± 4.1a |
| Large White 3 | 93.5 ± 2.9a | 38.3 ± 11.8b | 24.4 ± 2.6a | 121.2 ± 16.0a | 33.8 ± 5.6a | 73.3 ± 10.5a | 28.6 ± 8.8a | 47.6 ± 1.6a | 60.4 ± 2.1a |
| Large White 4 | 89.9 ± 3.7a | 44.0 ± 18.1b | 23.6 ± 6.6a | 137.7 ± 33.3a | 33.8 ± 8.9a | 86.7 ± 25.7a | 24.5 ± 1.2a | 39.4 ± 1.7a | 62.3 ± 5.1a |
| 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. abDifferent letters indicate significant differences (P<0.05).

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 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 between 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 × 10⁹ sperm cell/ml) as compared to the Hungarian Mangalica boar (0.490 × 10⁹ sperm cell/ml) (Egerszegi et al., 2008).
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.

REFERENCES

Agricultural Research Council (1993). Pig production in South Africa. Irene Animal Production Institute, Bulletin 427. V & R printers. Pretoria South Africa, pp. 19-97.

Alm K, Peltoniemi OA, Koskinen E, Andersson M (2006). Porcine field fertility with two different insemination doses and the effect of sperm morphology. Reprod. Domest. Anim. 41(3): 210-213.

Althouse GC, Kuster CE, Clark SG, Weisiger RM (2000). Field investigations of bacterial contaminants and their effects on extended porcine semen. Theriogenology, 53: 1167-1176.

Amann R (1989). Can the fertility potential of a seminal sample be predicted accurately? J. Androl. 10: 89-98.

Borg KE, Lunstra DD, Christenson RK (1993). Semen characteristics, testicular size, and reproductive hormone concentrations in mature Duroc, Meishan, Fengjing, and Minzhu boars. Biol. Reprod. 49: 515-521.

Chimonmyo M, Bhebhe E, Dzama K, Halimani TE, Kanengoni A (2005). Improving smallholder pig production for food security and livelihood of the poor in Southern Africa. Proc. African Crop Sci. Conference, 7: 569-573.

Egerszegi I, Sarlós P, Berger B, Tóth P, Rátky J (2008). Liquid and cryopreservation of Mangalica semen. Workshop on Conservation of traditional pig breeds with special regards to Mangalica, Herceghalom, Hungary 09. 12.

Flowers W (1997). Management of boars for efficient semen production. J. Reprod. Fertil. Suppl. 52: 67-78.

Food and Agricultural Organization (2007). Multiple chapters. In: Rischowsky B, Pilling D (eds), The state of the world’s animal genetic resources for food agriculture, Rome.

Frydrychová S, Čerňovský J, Lustyková A, Rozkot M (2010). Effects of long-term liquid commercial semen extender and storage time on the membrane quality of boar semen. Czech J. Anim. Sci. 55: 160-166.

Gadea J (2005). Sperm factors related to in vitro and in vivo porcine fertility. Theriogenology, 63: 431-444.

Halimani TE, Muchadeyi FC, Chimonmyo M, Dzama K (2010). Pig genetic resource conservation: The Southern African perspective. Ecolog. Econ. 69: 944-951.

Holt C, Holt W, Moore H, Reed H, Curnock R (1997). Objectively measured boar sperm motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials. J. Androl. 18: 312-323.

Hughes P, Varley K (1980). Reproduction in the pig: Fertility in the male, pp. 187-195.

Johnson LA, Weitzle KS, Fiser P, Maxwell WMC (2000). Storage of boar semen. Anim. Reprod. Sci. p. 62.

Kennedy BW, Wilkins JN (1984). Boar, breed and environmental factors influencing semen characteristics of boars used in artificial insemination. Can. J. Anim. Sci. 64: 833-843.

Kommisrud E, Paulenz H, Sehested E, Grevle I (2002). Influence of boar and semen parameters on motility and acrosome integrity in liquid boar semen stored for five days. Acta. Vet. Scand. 43: 49-55.

Kondracky S (2003). Breed differences in semen characteristics of boars used in artificial insemination in Poland. Pig News Information, 24(4): 119-122.

Kovács A, Foote RH (1992). Viability and acrosome staining of bull, boar and rabbit spermatozoa. Biotechnic. Histochem. 67: 119-124.

National Research Council (1998). Nutrient Requirement of Swine; National Research Council, National Academy Press, Washington, USA.

Ncube M, Dzama K, Chimonyo M, Kanengoni A, Hamudikuwanda H (2003). Effect of boar genotype on reproductive performance of the local sows of Zimbabwe. Livestock Research for Rural Development 15 (2). http://www.praissiesswine.com/pdf/34427.pdf.

Oh SH, See MT., Long TE, Galvin JM (2003). Genetic correlations between boar semen traits. J. Anim. Sci. 81: 317.

Prolit (2004). Pig Farming. Project Literacy Productions, Cape Town, South Africa.

Ramsay K, Harris L, Kotze A (comp./ed) (1994). Landrace breeds: South Africa’s indigenous and locally developed farm animals: Farm Animal Conservation Trust. Pretoria.

Rijsselaere T, van Soom A, Tanghe S, Coryn M, Maes D, de Kruif A (2005). New techniques for the assessment of canine semen quality: A review. Theriogenology, 64: 706-719.

Roeckl-MF (1996). Genetics and reproduction in the pig. Anim. Reprod. Sci. 42: 143-152.

Saacke RG (2008). Sperm morphology: Its relevance to compensable and uncompensable traits in semen. Theriogenology, 70: 473-478.

Salihun J, Thongtipsiridech S, Kornkaewrat K, Mahasawangkul S, Angkawanish T, Jansithiwate S, Boonprasert K, Pinyopummin A (2011). Practical Elephant Semen Analysis Techniques. EU-Asia Link Project Symposium “Managing the Health and Reproduction of Elephant Populations in Asia” 8-10 October, pp. 83-87.

Sanchez R, Barbosa J, Bellart A, Rius R, Garca P (1998). Effect of the percentage of semen morphological abnormalities in seminal doses on fertility and prolificity. Proc. 15th IPVS Congress, Birmingham, p. 71.

Scholtz MM (2005). The role of research and a seed stock industry in the in situ conservation of livestock genetic resources. In: Proc. of the 4th AACAA and TSAP Annual Meeting (20-24), pp. 313-316.

Snedecor GW, Cochran WG (1980). Statistical Methods, Seventh edition. Iowa. State University Press. I. D. Hill.

Swart H, Kotze A, Olivier PAS, Grobler JP (2010). Microsatellite-based characterization of Southern African domestic pigs (Sus scrofa domestica). S. Afr. J. Anim. Sci. 40(2): 121-132.

Tardiff S, Laforest JP, Cormier N, Bailey J (1999). The importance of porcine sperm parameters on fertility in vivo. Theriogenology, 52: 447-459.

Tretipskul C, Buranaamnuay K, Koonjaenak S, Tummaru P, Techakumphu P (2010). The Use of Computer-Assisted Sperm
Analysis for discriminating series of motility pattern of frozen-thawed boar semen. Thai J. Vet. Med. 40(1): 25-30.

Waberski D, Meding S, Dirksen G, Weitze K, Leiding C, Hahn R (1994). Fertility of long term stored boar semen: influence of extender (Androhep and Kiev), storage time and plasma droplets in the semen. Anim. Reprod. Sci. 36: 145-151.

Walters AH, Eyestone WE, Saacke RG, Pearson RE, Gwazdauskas FC (2005). Bovine embryo development after IVF with spermatozoa having abnormal morphology. Theriogenology, 63: 1925-1937.

Wolf J, Smital J (2009). Effects in genetic evaluation for semen traits in Czech Large White and Czech Landrace boars. Czech J. Anim. Sci. 54: 349-358.

Xu X, Pommier S, Arbov T, Hutchings B, Sotto W, Foxcroft G (1998). In vitro maturation and fertilization techniques for assessment of semen quality and boar fertility. J. Anim. Sci. 76: 3079-3089.