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Comparison of different extenders and storage temperature on the sperm motility characteristics of Kolbroek pig semen

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Abstract

Maintaining a successful pig artificial insemination programme depends on a number of factors, including evaluation of semen characteristics. This study compared the efficacy of different extenders on the sperm motility of Kolbroek semen during short term storage at 4 °C and 25 °C. Semen was collected from Kolbroek boars using the gloved hand technique and transported to the laboratory for evaluation. Semen was pooled and randomly allocated to four groups and diluted at a ratio of 1:1 (v/v) with Beltsville thawing solution (BTS), Kobidil^{+}, egg yolk citrate (EYC) and non-extended semen (Control). Each extender had two similar semen samples, making a total of eight samples. Extended and non-extended semen were stored at 4 °C and the other samples at 25 °C for 1 h. Data were analyzed using analysis of variance (ANOVA). The total sperm motility of semen stored at 25 °C was higher when semen was extended with BTS and Kobidil^{+} in comparison to the egg yolk citrate diluent. However, total sperm motility in the non-extended semen did not differ from the BTS and EYC group during storage at 25 °C. Sperm progressive motility was higher in the BTS group, compared to the Kobidil^{+} and non-extended groups. Sperm motility of Kolbroek semen at 4 °C did not differ between all extender treatments. Total motility rate was significantly higher when Kolbroek sperm were stored at 25 °C than at 4 °C. It can be concluded that Kolbroek sperm, extended with BTS, maintained their motility rate better for short term storage at 25 °C in comparison to 4 °C.

Keywords: Indigenous pigs, sperm, extenders, pig, Kolbroek
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Introduction

The Kolbroek is a unique indigenous South African pig breed. It is hardy, seemingly well adapted to harsh environmental conditions and maintaining reproduction. Due to its unique gene pool, it is imperative to study its sperm characteristics to conserve the breed's genetic material for future breeding and research purposes. Furthermore, it has been documented in other studies of a variation between and within individual male breeds in sperm traits (Kiso *et al*., 2011; Masenya *et al*., 2012).

Several extenders have been developed to preserve pig semen for long and short term storage. Semen extenders protect sperm against seminal plasma which can be toxic, while providing temperature protection for sperm by reducing its metabolic rate in low or chilling temperatures during storage. This allows multiple inseminations from a single ejaculate and provides membrane stabilization during cooling. It has been reported that extendera can act as an energy source for sperm metabolism, can provide pH buffering from sperm cell waste, ions for membrane and cell balance, and antibiotics to prevent growth of microbes that can cause diseases (Knox, 2011). Semen extenders also provide nutrients needed for the metabolic maintenance of sperm to perform their function. For this reason antibiotics and sugars are added to the extender (Otite, 2000; Gadea, 2003; Orok *et al*., 2010). Frydrychova *et al*. (2010) reported that extenders are extensively used to preserve not only sperm viability, but also the sperm motility rate for an optimal period of time.

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It is well-known that for *in vitro* semen preservation, a good extender should also provide an environment that inhibits the formation of harmful reactive oxygen species (ROS) or lipid peroxide (Orok *et al*., 2010) and cold shock. Reactive oxygen species damages sperm membrane and DNA which in turn reduces the sperm's motility rate and ability to fuse with the oocyte which compromises the paternal genomic contribution to the embryo (Boonsorn *et al*., 2010). When ram, bull and boar semen are cooled to temperatures below 15 °C, there is an irreversible loss in sperm motility and metabolic activities (Orok *et al*., 2010). Beltsville thawing solution (BTS) and Kobidil^{+} are commercial short term extenders. BTS was first developed for the cryopreservation of semen and the protocol has subsequently been modified to accommodate fresh pig semen at 15 to 18 °C (Pursel & Johnson, 1975). On the other hand, egg yolk citrate (EYC) is known to minimize the adverse effect of low temperatures and provide protection to the sperm during cooling (Katila *et al*., 1997). Furthermore, some extenders have been shown to increase semen storage time for up to 3 days (Johnson *et al*., 2000), and even 5 to 7 days (Levis, 2000).

Preserving pig semen for short or prolonged period requires storage at low temperatures (15 - 18 °C) in which the use of refrigerators equipped with a thermostat for accurate temperature adjustment can be provided (Correa *et al*., 2006). However, in an environment where the temperature is high throughout the year, such equipment may not be able to maintain the set temperature (Correa *et al*., 2006). Keeping pig semen in cooled temperature around 5 ºC was reported to be a cheaper alternative than liquid nitrogen to help with increasing the use of artificial insemination in the pig industry. Another benefit is that bacterial growth is reduced at 5 ºC, which would improve the quality of semen. However, pig semen is known to be very sensitive to low temperature. Therefore, an assessment of indigenous Kolbroek sperm quality in different extenders at low or high temperature is important to determine which extender to use and what temperature will be suitable for preservation or short-term storage.

The objective of this study was thus to compare the efficacy of different extenders during the preservation of Kolbroek pig semen at 4 °C and 25 °C.

Materials and Methods

This trial was conducted at the Agricultural Research Council, Irene. South African indigenous Kolbroek boars, ranging from 9 to 10 months of age, were used in the study. The boars were housed in individual cages for routine semen collection (Morrell & Wallgren, 2010). Semen was collected from three Kolbroek boars of proven good semen quality on five occasions. At each event ejaculates were collected from each boar by the gloved hand technique (Roca *et al*., 2004). The sperm-rich fraction was collected using a thermo flask containing warm water (39 ºC) and a glass beaker covered with a gauze filter to separate the gel fraction from the sperm-rich fraction (Roca *et al*., 2004). Within an hour of collection, semen was transported to the laboratory for the evaluation of sperm motility characteristics. The collected semen was pooled and divided into a total of eight 10 mL centrifuge tubes, i.e. two tubes per treatment in 4 x 2 factorial experiments using a randomised block design. Thus, two tubes of semen samples per extender were diluted in a ratio of 2:2 (v/v) with either (i) Beltsville Thawing Solution (BTS), (ii) Kobidil^{+}, (iii) egg yolk citrate (EYC) and (iv) not-diluted semen (control, not-extended). After dilution, four samples of the different treatments were then placed in a walk-in cool room, set at a temperature of 4 °C. The other four semen samples were placed in a controlled room temperature laboratory at 25 °C and equilibrated for an hour interval. Following equilibration, all eight samples from 4 °C and 25 °C were evaluated using the swim-up (10 µL of semen was added to 500 µL of swim up media) method. Sperm motility characteristics were analyzed by the Sperm Class Analyzer (SCA, Masenya *et al.*, 2012). Then 10 µL of each treated Kolbroek semen sample was placed in a 10mL centrifuge tube containing 500 µL Bracket and Oliphant's sperm-wash media and co-incubated at 39 °C for 5 min. After incubation, a drop (10 µL) of Kolbroek semen from each treatment was placed on a microscopic slide and evaluated microscopically for sperm motility rate, with the aid of sperm class analyzer. The sperm motility characteristics included: total motility TM), progressive movement (PM), non-progressive movement (NPM), rapid (R), medium (M), slow (S) and static (immovable) sperm.

Data was analyzed using the program GenStat. Analysis of variance (ANOVA) was used for analyses. Means of different treatments were separated using Fisher's protected t-test least significant difference (LSD) at 5% level of significance (Snedecor & Cochran, 1980).

Results and Discussion

Sperm motility characteristics of Kolbroek semen stored at 25 °C were higher (P <0.05) than those stored at 4 °C, when semen was extended with BTS and Kobidil$^+$, compared to the egg yolk citrate extender (Table 1). However, total sperm motility rate of semen that was not extended did not differ (P >0.05) from BTS and EYC during storage at 25° C. Furthermore, the number of sperm with no progressive movement did not differ (P >0.05) in all treatments. Sperm showing progressive movement were higher (P <0.05) in semen extended with BTS, compared to Kobidil$^+$ and non-extended semen. However, there were no differences (P >0.05) recorded in the motility characteristics of the Kolbroek pig sperm in all treatments when stored at 4 °C. Moreover, the percentage of total sperm motility observed was low when semen was stored at 4 °C. Highest immotile (static) sperm was recorded in semen stored at 4 °C regardless of the extender used.

Table 1 Mean (± SE) motility characteristics of Kolbroek pig sperm stored at 25 °C or 4 °C in different extenders

Sperm motility characteristics	Storage temperature	Extender treatments			
		BTS	Kobidi$^+$	EYC	NE
Total motility	25 °C	$71.3^{ab} \pm 0.5$	$78.8^{a} \pm 4.9$	$41.9^{bc} \pm 17.7$	$64.1^{abc} \pm 2.9$
	4 °C	$19.3^{e} \pm 5.3$	$10.9^{e} \pm 4.5$	$15^{e} \pm 2.7$	$2.0^{e} \pm 0.4$
N-progressive	25 °C	$44.3^{a} \pm 1.5$	$45.1^{a} \pm 1.3$	$24.3^{a} \pm 15.1$	$43.1^{a} \pm 1.3$
	4 °C	$7.4^{e} \pm 1.8$	$6.4^{e} \pm 2.1$	$8.4^{e} \pm 1.3$	$2.1^{e} \pm 0.4$
Progressive	25 °C	$27.0^{a} \pm 0.9$	$33.8^{ab} \pm 6.2$	$17.7^{c} \pm 2.5$	$21.1^{d} \pm 1.5$
	4 °C	$11.9^{e} \pm 3.4$	$4.5^{e} \pm 2.3$	$6.6^{e} \pm 1.3$	$0.0^{e} \pm 0.0$
Rapid	25 °C	$35.3^{a} \pm 0.1$	$46.4^{a} \pm 11.6$	$24.2^{a} \pm 16.5$	$32.5^{a} \pm 0.3$
	4 °C	$8.0^{e} \pm 0.3$	$6.4^{e} \pm 4.2$	$9.1^{e} \pm 0.6$	$1.2^{e} \pm 0.0$
Medium	25 °C	$16.6^{a} \pm 1.1$	$11.5^{a} \pm 0.1$	$11.8^{a} \pm 2.7$	$15.1^{a} \pm 0.1$
	4 °C	$6.2^{e} \pm 3.0$	$2.7^{e} \pm 0.5$	$3.0^{e} \pm 0.5$	$0.0^{e} \pm 0.0$
Slow	25 °C	$19.4^{a} \pm 0.6$	$20.9^{a} \pm 6.6$	$5.9^{a} \pm 1.6$	$16.4^{a} \pm 2.9$
	4 °C	$5.1^{e} \pm 2.5$	$1.8^{e} \pm 0.7$	$2.9^{e} \pm 1.4$	$0.8^{e} \pm 0.4$
Static	25 °C	$28.7^{a} \pm 0.5$	$21.2^{ab} \pm 4.9$	$58.1^{a} \pm 17.6$	$35.9^{a} \pm 2.8$
	4 °C	$80.7^{e} \pm 5.3^{a}$	$89.1^{e} \pm 4.4$	$85.1^{e} \pm 2.6$	$98.0^{e} \pm 0.4$

^{abcde} Means within rows with different superscripts differ significantly (P <0.05).
Beltsville thawing solution (BTS), Egg yolk citrate (EYC), Non-extended semen (NE), Total motility (TM), Non-progressive (N-progress) Progressive (Progress).

The present study demonstrated that the type of semen extender used had an effect on motility characteristics of Kolbroek sperm during storage at 4 or 25 °C. Kolbroek semen diluted with the Beltsville thawing solution or Kobidil$^+$ extender and stored at 25 ºC showed a higher total sperm motility rate, compared to sperm in egg yolk citrate, i.e. 71.3% and 78.8% vs. 41.9%. It was further observed that, the progressive motility rate measured by Computer Aided Sperm Analysis (CASA), known as Sperm Class Analyser (SCA), was (P <0.05) higher when Kolbroek semen was extended with BTS (27%) and Kobidil$^+$ (33.3%) compared to the other extenders studied. These results are supported by the findings of Vyt *et al.* (2004) where Beltsville thawing extender and Kobidil$^+$ were found to be acceptable extenders for the short term liquid preservation of pig semen. Moreover, they preserved sperm motility better than the Androhep extender (Vyt *et al*., 2004).

However, these findings differ from the findings of Rienprayoon *et al*. (2012), who found that boar semen diluted with ModenaTM extender had a higher sperm motility rate compared to that obtained with BTS. This may be due to that ModenaTM extender containing an antioxidant which aided in the reduction of the occurrence of reactive oxygen species (ROS) during the holding period. It was well established that ROS

can damage the sperm plasma membrane and hence reduced the sperm motility rate (Guthrie & Welch, 2005; Chanapiwat *et al*., 2009).

The effect of temperature on Kolbroek sperm motility rate was also evident from the total motility rate. Sperm motility rate differed (P <0.05) for sperm stored at 4 and 25 ºC. Total motility rate of Kolbroek sperm stored at 25 ºC in the different extenders ranged from 41% to 78%, compared to a sperm motility rate of Kolbroek semen preserved at 4 ºC (2.0% to 19.3%). Furthermore, a temperature of 4 ºC caused a rapid decrease in total motility rate following one hour of storage. This is in agreement with the results obtained by Zou & Yang (2000) where storage of semen at 4 ºC reduces sperm motility in pigs. In this study, Kolbroek sperm has less tolerance to 4 °C storage or equilibration. It has previously been shown that boar spermatozoa are particularly susceptible to cold shock when exposed to temperatures below 15 ºC (Zou & Yang, 2000).

Conclusion

It can be concluded that Kolbroek pig sperm maintained their motility rate more satisfactory when stored for a short-term at 25 °C than at 4 °C, with BTS used as an extender. Therefore, Kolbroek sperm can be stored at 25 °C and maintain acceptable motility rate before artificial insemination is performed.

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