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Research Application Summary

Characterization of semen from the unimproved South African indigenous goats

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Abstract

In this study, we characterised semen of the unimproved South African (SA) indigenous goat with an aim of determining the potential of using modern techniques such as artificial insemination to multiply it. The SA unimproved goat is hardy and adaptable to the local harsh environment and is now attracting attention of commercial farmers. The average percentages of normal and live sperms (79% and 76.3%, respectively) for this goat fell within the range of good quality sperms. Morphological evaluations of the sperms also revealed that these goats had normal sperms, with abnormalities falling within the normal range for good quality sperms. Similarly sperm motility was also normal. From this study we conclude that the unimproved SA indigenous goat has potential to be multiplied and commercially produced using modern techniques.

Key words: Artificial insemination, cryopreservation, sperm morphology, sperm quality, sperm viability

Résumé

Dans cette étude, nous avons caractérisé le sperme de la chèvre indigène non améliorée d'Afrique du Sud (SA) dans le but de déterminer le potentiel d'utilisation de techniques modernes telles que l'insémination artificielle pour la multiplier. La chèvre non améliorée de SA est rustique et adaptable à l'environnement local rude et attire actuellement l'attention des agriculteurs commerciaux. Les pourcentages moyens de spermatozoïdes normaux et vivants (respectivement 79% et 76,3%) pour cette chèvre sont compris dans la gamme des spermatozoïdes de bonne qualité. Les évaluations morphologiques des spermatozoïdes ont aussi révélé que ces chèvres avaient des spermatozoïdes normaux, avec des anomalies se trouvant dans la gamme normale de spermatozoïdes de bonne qualité. De même la motilité des spermatozoïdes était également normale. De cette étude, nous concluons que la chèvre indigène non

améliorée de SA a la possibilité d'être multipliée et d'être commercialement produite en utilisant des techniques modernes.

Mots clés: Insémination artificielle, congélation, morphologie des spermatozoïdes, qualité du sperme, viabilité du sperme

Background

Goats play an important socio-economic role of providing meat, milk and income. The unimproved South African indigenous goats have generally been farmed by the majority of the small scale farmers in the rural areas of South Africa (S.A) for many years. The growth rate of these goats however is generally low, although the breed seems to be very fertile and adapted to the local harsh conditions (Schwalbach and Greyling, 2000). This goat breed has previously received little attention from researchers. As a result little effort has been made to genetically improve it like the other indigenous (improved) S.A breeds (e.g. Boer, Kalahari Red and Savannah goats) that enjoy international recognition (Schwalbach and Greyling, 2000).

However, the unimproved South African indigenous goat is now enjoying increasing interest from commercial farmers, due to its hardiness and adaptability to the local environmental conditions (Webb and Mamabolo, 2004; Sundararaman and Edwin, 2008). Very little is known about the semen quality of the Unimproved South African indigenous goats and its tolerance to cryopreservation. It is therefore important to further study the physiology of this breed specifically semen quality and important reproduction related parameters, so as to improve the breed through selection. Currently little has been done to characterize its reproductive traits.

Literature Summary

Sperm motility is commonly believed to be one of the most important criteria used, when evaluating the fertility potential of semen (Hashida and Abdullah, 2003). It has also been stated that the sperm motility characteristics of goat semen can be useful in the selection and ranking of bucks regarding their fertility and an indicator of sperm quality (Sundaraman and Edwin, 2008). Yamashiro *et al*. (2006) considered a buck semen ejaculate of 0.75 ml, a sperm motility of more than 80% and concentration of more than 3×10^9 sperm/ml to be of high quality. In addition, mammalian semen is quoted to normally have a pH in the range of 7.2 to 7.8 (Prins, 1999). The aim of this study was thus to characterize the semen of the unimproved South African indigenous goat and to provide baseline information for future

studies regarding the preservation (refrigeration or cryopreservation) of semen for AI utilization.

Study Description

This study was conducted at the Agricultural Research Centre (ARC), Irene campus (25° 55' S; 28° 12' E), Republic of South Africa (RSA), located in the highveld region of RSA, situated at an altitude of 1525m above sea level. The climatic conditions and ambient temperatures range from hot days to cool nights in summer to moderate winter days with cold nights and an average annual rainfall of 464mm (Webb *et al*., 2004).

A total of 10, young mature unimproved indigenous goat bucks (mean body weight 38.1 ± 9.3 kg; 2 to 3 years of age) were trained for semen collection using the artificial vagina (AV) method. The trial was conducted between April (autumn) and August (winter), 2009. During this period the bucks grazed on natural pastures and received fresh water *ad libtum.* Additional supplementation (300–350 g concentrate or 1kg maize silage/ animal/day - depending on the availability) was also provided. Experimental animals were cared for and handled in accordance with standard protocols and guidelines for the Care and Use of Animals of the Agricultural Research Council.

Bucks were trained to mount and ejaculate in the AV daily (7 days a week), before the onset of the study. Training for semen collection by the AV method was executed for a period of 4 weeks, using a doe in oestrus as a teaser for semen collection. Briefly, a female was detected in oestrus and restrained in a neck clamp before the introduction of the buck into the testing arena or pen (Silvestre *et al.*, 2004; Bester, 2006). In order to improve the libido of the bucks these animals were placed in a pen adjacent to the semen collection arena prior to semen collection, so that the bucks were able to observe the other males mounting the restrained doe and be sexually stimulated (Price *et al*., 1984; Silvestre *et al.*, 2004). Bucks were allowed a 5 minute period to attempt to mount and ejaculate. After ejaculation or a period of 5 minutes, whichever occurred first, the buck was transferred to the adjacent pen and after 10 to 15 minutes, males that did not ejaculate at their previous attempt, were again placed in the semen collection arena. Training was considered successful when males mounted and ejaculated into the AV at regular intervals i.e. 4 consecutive days when presented to any restrained doe as a teaser, even if the doe was not in oestrus, in the presence of the same semen collector (Silvestre *et al*., 2004).

Seven South African unimproved indigenous bucks that were successfully trained for semen collection with the aid of an artificial vagina (AV) were used in this study. Semen was collected from each buck twice weekly (Gacitua and Arav, 2005; Sundararaman and Edwin, 2008) with the aid of an AV filled with water at 42 - 46°C (Silvestre *et al.*, 2004; Yamashiro *et al*., 2006). Six ejaculates were obtained from each buck in a period of 3 weeks between April and May 2009 (during the natural breeding season). During the semen collection process, when the buck mounted a doe, the penis was gently guided into the AV. To minimize stress and maximize the quality of the ejaculates, collections were always carried out under the same conditions, i.e. by the same person, at the same time of the day (8:00 – 10:00), in the same pen and using the same equipment.

Immediately following semen collection, the ejaculate was macroscopically evaluated and transferred into plastic screw top conic tubes, placed and stored in a thermo-flask and maintained at 37 °C (Silvestre *et al*., 2004), until sperm assessment (electronic/automated and microscopic analyses) at the Germplasm, Conservation and Reproduction Biotechnologies Laboratory (GCRB) of the ARC, within 1h (Gacitua andArav, 2005).

The semen volume was determined using a graduated collection tube and recorded immediately after collection. The semen pH was then measured manually with the aid of Neutralit® pH-indicator strips (Merck KGaA), by pippeting a drop (10 µl) of fresh semen onto the strip and spreading it gently. The resultant colour of the strip was then compared to the colour code of the graduated pH scale, to obtain a pH reading. In the laboratory, the sperm concentration of the semen was determined with the aid of a SpermaCue®. A volume of 20 µl undiluted fresh semen was pipetted into a microcuvette which was then inserted into the SpermaCue to give an automated sperm concentration reading in $X10^6$ sperm/ml.

Sperm viability (percentage live sperm) was determined using the new improved nigrosin/eosin (N/E) stain (pH=8.39; osmolarity=411), mixed in the ratio of 60 µl eosin/nigrosin and 6 µl semen aliquots. The vital staining method used was indicative of the percentage live or dead status of the sperm cells and also allowed a good evaluation of the sperm morphology (normal or abnormal) (Bjorndahl *et al*., 2003). When using this staining technique, live sperm fluoresce white, while the dead sperm

colour red by absorbing the stain (Bearden *et al*., 2004). The live sperm were further categorized into morphologically normal or abnormal sperm and the abnormalities were recorded using two different sets of criteria. The first criteria used the location of the abnormality such as head, mid-piece and tail. Abnormalities were then also classified as primary, secondary or tertiary, according to the nature of the lesion (Loskutoff and Crichton, 2001).

For automated sperm analyses, a Sperm Class Analyzer®-SCA® system was used to analyze the sperm motility (percentage immotile/static sperm or motile, but not progressive and progressively motile sperm) and certain velocity parameters (static, slow, medium, rapid, curvilinear, straight-line, average path, linearity, straightness and wobble velocities). A total volume of 10 µl of semen was diluted with 500 µl of BO medium for swim-up and incubated in the MCO-20 AIC Sanyo CO_2 incubator (Sanyo Electric Biomedical Co.,Ltd, Japan), adjusted to 37°C for 5 minutes. Following an incubation period, 5 µl of this semen solution was pipetted onto pre-warmed, bevel-edged, frosted-end, microscope glass slides (Thermo Scientific Menzel-Gläser), gently covered with a microscope cover glass (Menzel gläser, Germany) and evaluated under x10 magnification of the CASA microscope, from the image on the monitor.

Data were analyzed using the statistical program GenStat® 2003 and are presented as the mean and standard deviation (SD), for each buck (for 6 collections over a 3 week period). The overall mean (± SD) for all parameters considered were also calculated. Analysis of variance (ANOVA) for unbalanced data was used to compare the semen from the different unimproved indigenous bucks in terms of semen volume, semen pH, sperm concentration, morphology and motility. The data were normally distributed with homogeneous treatment variances, and comparisons were done at the 5% level of confidence.

Research Application

In general, the mean values observed for semen volume and pH, as well as for sperm concentration, were similar for all the experimental animals used - with an exception of one buck which produced a significantly higher ejaculate volume than any of the other animals evaluated. This animal however recorded similar semen pH and sperm concentration values compared to all other bucks evaluated in the trial.

The South African unimproved indigenous bucks recorded an overall average percentage of 79.0 ± 6.3% for normal and 76.3

± 8.2% for live sperm, which comply with the mean for good quality semen (Gil *et al*., 2001).

Similar means were recorded for all the different types of sperm abnormalities (head, mid-piece and tail) in all the unimproved indigenous goats evaluated. This indicated that all bucks used had a similar sperm morphology, which gives credibility to the overall average abnormalities obtained in this study.

The primary, secondary and tertiary sperm abnormalities for all the study animals were also similar. Similarly, the sperm motility values did not differ much among bucks as determined by the CASA system. The static sperm and total sperm motility for the bucks was 31% and 69%, respectively.

Recommendation

South African unimproved indigenous bucks have been shown to produce a lower semen volume, sperm concentration, and less progressively motile sperms, compared to the European, Asian breeds and also the improved S.A Boer goat. Although this was a preliminary study, results show that the unimproved indigenous breed has potential for multiplication through modern breeding techniquest such as A.I.

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