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Computer assisted sperm analysis of motility patterns of postthawed epididymal spermatozoa of springbok (*Antidorcas marsupialis*), impala (*Aepyceros melampus*), and blesbok (*Damaliscus dorcus phillipsi*) incubated under conditions supporting domestic cattle in vitro fertilization

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Abstract

The need for information on the reproductive physiology of different wildlife species is important for ex situ conservation using such methods as in vitro fertilization (IVF). Information on species reproductive physiology and evaluation of sperm quality using accurate, objective, repeatable methods, such as computer-assisted sperm analysis (CASA) for ex situ conservation has become a priority. The aim of this study was to evaluate motility patterns of antelope epididymal spermatozoa incubated for 4 h under conditions that support bovine IVF using CASA. Cauda epididymal spermatozoa were collected postmortem from testicles of springbok (N = 38), impala (N = 26), and blesbok (N = 42), and cryopreserved in biladyl containing 7% glycerol. Spermatozoa were thawed and incubated in Capacitation media and modified Tyrode lactate (m-TL) IVF media using a protocol developed for domestic cattle IVF. The study evaluates 14 motility characteristics of the antelope epididymal sperm at six time points using CASA. Species differences in CASA parameters evaluated under similar conditions were observed. Several differences in individual motility parameters at the time points were reported for each species. Epididymal sperm of the different antelope species responded differently to capacitation agents exhibiting variations in hyperactivity. Motility parameters that describe the vigor of sperm decreased over time. Spermatozoa from the different antelope species have different physiological and optimal capacitation and in vitro culture requirements. The interspecies comparison of kinematic parameters of spermatozoa between the antelopes over several end points contributes to comparative sperm physiology which forms an important step in the development of species specific assisted reproductive techniques (ARTs) for ex situ conservation of these species.

Keywords: Antelope; Protocols; Ex situ conservation

1. Introduction

The loss of biodiversity on wildlife populations is a cause for concern to conservationists worldwide. Anthropogenic practices that cause loss of wildlife diversity, genetic variability, and population decline in wild

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animals include population fragmentation through land transformation, habitat fragmentation, and alteration resulting in habitat loss, excessive consumptive utilization and predation [1–5]. To limit the loss of biodiversity and the negative effect of small and fragmented populations, there is an urgent need for the development and use of all means available which may include the use of ex situ procedures to conserve species outside their natural habitat. This includes the use of captive breeding programs and assisted reproductive techniques (ARTs) to preserve and use gametes and genomic material from different species. This initiative has become progressively important, considering the possibilities that this approach offers. Conservation efforts that promote genetic variability in both in situ and ex situ populations are therefore highly recommended [6]. A large component of assisted reproductive technique conservation is dependent on knowledge of the reproductive biology and physiology of different species and use of reproduction protocols for the transfer of genetics [5,7–9]. Therefore, this research will evaluate sperm quality and function based on the evaluation of sperm motility parameters using computer assisted sperm analyzer by means of the sperm class analyzer (CASA-SCA).

Motility is the most commonly used parameter in the evaluation of frozen sperm [10] and predicting the fertilizing capacity of animals [11,12]. Sperm motility reflects several essential aspects of sperm metabolism, and is a readily assayed barometer of relative cell health [13]. In equine, longevity of motility evaluated using light microscopy has been significantly correlated with the foaling rate [14]. Some authors suggest that conventional sperm analysis is sufficient to evaluate the fertility potential of semen [12]. However conventional evaluation which is based on subjective visual measurements may underestimate good motility in some species [13]. Studies have also demonstrated a large variation between and within technician in evaluating motility. This has resulted in inaccurate and imprecise measurements of sperm motility [15] and a considerable variation in sperm quality measurements amongst laboratories, processing methods, and microscopic analysis of sperm samples. This makes data incomparable, and interpretation of results complex [16] particularly when working with wildlife samples. These limitations imply that conventional motility should be evaluated together with other parameters to obtain a better estimate of sperm fertilizing potential [17–19]. Computer assisted sperm analysis (CASA) therefore provides a more reliable accurate and objective method of evaluating motility while facilitating a rapid analysis of motility patterns. CASA systems, such as the SCA can greatly assist to standardize methodologies.

The SCA produces comprehensive kinematic data for sperm motility which could be used to define sperm which vary in their motility track or trajectory [20,21] providing comprehensive information on sperm quality in terms of motility than just overall motility. The SCA can therefore be used to monitor changes in motility patterns over time thus giving insight into the physiological characteristics of sperm in different in vitro environments. In an effort to contribute to ex situ conservation of selected South African antelope and understanding the physiology of their sperm, the aim of this study is therefore to evaluate changes in motility patterns of postthawed epididymal sperm subjected to a protocol that supports domestic cattle in vitro fertilization (IVF) by means of CASA-SCA.

2. Materials and methods

Chemicals used in this study were obtained from Sigma, South Africa unless otherwise stated.

2.1. Testes collection and sperm extraction

Testes were collected postmortem from sexually mature, free ranging springbok (N = 38), impala (N = 26), and blesbok (N = 42) during organized winter culls in the period May to July in South Africa. Animals were obtained in their breeding season from different game farms and reserves throughout South Africa. Culls or hunts were conducted or supervised by professional hunting teams or staff. None of the animals had been born captive or hand-raised. All animals were killed for management purposes on the game farms or reserves. The paired testes from each animal were removed and transported for sperm processing in closed plastic bags to an onsite mobile laboratory within 3 h of death in a cooled styrofoam container. Testes were placed on cardboard under which were ice bricks avoiding contact with ice bricks but maintaining a temperature of between 4 °C and 5 °C. The epididymis and proximal vas differentia were detached from both testes. The cauda epididymis and vas deferens from each animal were detached from the caput and corpus epididymis. A blunt 26-gauge needle was inserted into the lumen of the vas deferens for sperm harvesting. The needle was attached to a syringe containing 2 mL of Biladyl A extender (Minitüb, Tiefenbach, Germany) consisting of 2.42% Tris, 1.38% citric