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Classification of ostrich sperm characteristics

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ABSTRACT

The success of assisted reproduction techniques is dependent on a sound foundation of understanding sperm characteristics to evaluate so as to improve semen processing. This study offers a descriptive basis for ostrich semen quality in terms of sperm function characteristics (SFC) that include motility, measured by computer assisted sperm analysis CASA (SCA®), viability (SYBR14/PI) and membrane integrity (hypo-osmotic swelling test). Relationships among these SFC's were explored and described by correlations and regressions. Certain fixed effects including the dilution of semen, season, year and male associated with semen collection were interpreted for future applications. The seasonal effect on sperm samples collected throughout the year suggested that it is prudent to restrict collections to spring and summer when SFC's and sperm concentration are maximized, compared to winter when these aspects of sperm quality are suppressed. Dilution of ejaculates helped to maintain important SFC's associated with fertilization success. The SFC's and sperm concentration varied among males, with specific males, having greater values for the percentage of motile (MOT) and progressively motile (PMOT) sperm, as well as sperm velocity (VCL, VSL, VAP) and linearity (LIN) variables. Males may thus be screened on these variables for inclusion in an artificial insemination (AI) programme to optimize fertility success rates.

1. Introduction

Variation in semen quality in terms of functionality within and between species, males as well as ejaculates, has been well documented (Songsasen and Leibo, 1997; Blanco et al., 2000; King et al., 2000; Yu et al., 2002; Blesbois et al., 2005; Roca et al., 2006; Chaveiro et al., 2006; Leahy and Gadella, 2011). Because of inter- and intra-male variation in ejaculate quality, semen samples should be evaluated before processing for storage and AI. The initial ejaculate quality is of utmost importance for successful semen processing because sperm cells are irreparable (Blesbois et al., 2005; Graham and Moce 2005). Damage that is likely to occur during processing will lead to a decrease in sperm function after storage, manifested more explicitly in cryopreserved semen than in chilled or neat semen. A 40% to 70% reduction in different sperm functions have been reported in the literature for cryopreserved sperm of both domestic and non-domestic avian species, emphasizing the importance of an ejaculate with good initial quality (Parks and Graham, 1992; Donoghue and Wishart, 2000; Watson, 2000; Gee et al., 2004; Malecki et al., 2008; Moce et al., 2010). In the ostrich, semen cryopreservation has

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been attempted by Malecki and Kadokawa (2001) and liquid storage has also been assessed by Ya-jie et al. (2001), but with limited success. Malecki and Kadokawa (2001) reported a mean of 11 ± 1% and Ya-jie et al. (2001) a mean of 26.1 ± 10.1% overall live sperm.

Semen processing technology can be technical, costly and time consuming and should thus not be wasted on a poor quality semen sample. Assessing semen throughout the processing protocol can also give an indication of the type and amount of damage exerted on the cell during the different stages and can be used as a basis for protocol optimizations.

Poor sperm production and supply has been noted as one of the primary reasons for poor fertility in the ostrich industry and has stressed the importance of effective male fertility evaluation (Bertschinger et al., 1992; Hemberger et al., 2001; Malecki and Martin, 2003; Malecki et al., 2008). The evaluation and selection of males for semen quality and potential fertility is a very important factor to consider before including a male in a breeding scheme (natural or artificial reproduction, stored or non-stored). Knowledge of the capacity of an ostrich male to contribute to an artificial insemination (AI) programme would allow the timely exclusion of males with inferior sperm quality. The maintenance of a resource population for AI is a costly and hazardous practice that includes many challenges. The ratio of males to females kept in a natural reproduction scheme, where a colony breeding system is most prevalent, can also be reduced with greater knowledge of the male's sperm quality (Lambrechts, 2004). The latter will potentially increase overall profitability by increasing chick numbers while maintaining fewer males with greater sperm functional quality.

Recent advances in ostrich semen collection by means of the "dummy-female" method developed by Rybnik et al. (2007) facilitated obtaining representative biological ejaculates, suitable for evaluation. Ejaculate quality was not compromised at a collection frequency of up to two times per day (Bonato et al., 2011). Ejaculate quality can, therefore, be assessed according to different sperm functional tests developed as adapted specifically for ostrich by Smith (2016). Sperm functional tests have been well correlated with sperm survivability after storage and acceptable fertility after AI in most other species, including men (Mahmoud et al., 1998), bulls (Ericsson et al., 1993; Farrell et al., 1998; Kasimanickam et al., 2006), roosters (Wishart and Palmer, 1986) and turkey toms (King et al., 2000). Subjective visual measures of conventional semen variables (commonly used to evaluate sperm variables in various livestock industries) are not highly repeatable or reliable when predicting fertility and are thus not recommended (Linford et al., 1976; Neuwinger et al., 1990; Hoflack et al., 2005; Moce and Graham, 2008). Sperm function variation can, therefore, be used to develop an objective, cost effective, time efficient and reliable classification system for objective evaluation of ostrich ejaculates and male screening. The aim of the present study was, thus, to describe the variation of functional sperm variables within and among ostrich ejaculates.

2. Material and methods

2.1. Animal population

Ten South African Black (SAB) ostrich males (*Struthio camelus var. domesticus*), aged between 3 and 7 years, were allocated to the study over a period of 5 years (2011–2015), although ejaculates collected in 2013 and 2014 were primarily used. Ejaculates ($n = 326$) were collected from these males using the "dummy" female method as described by Rybnik et al. (2007). Briefly, the dummy was made of hemp sack that inside had a steel frame structure cushioned with dense foam, providing firm support for the male chest and leg, and the PVC tube to which the artificial cloaca was inserted. Ejaculates were collected during winter (June to August), spring (September to November), and summer (December to February). Males in the resource population were screened from the commercial ostrich breeding flock, maintained at the Oudtshoorn Research Farm situated in the Klein Karoo, South Africa region (33°63′ S, 22°25′ E), on the basis of behavioural attributes rendering them suitable for AI (referred as desirable behaviour as described by Bonato et al., 2013). The origin of the ostrich flock and the general management procedures implemented therein were described previously (Van Schalkwyk et al., 1996; Bunter and Cloete, 2004).

2.2. Semen preparation

Ejaculates were diluted 1:1 (Malecki and Kadokawa, 2001; Sood et al., 2012) after collection with the ostrich specific diluent (OS1) developed by Smith (2016). The OS1 diluent content was based on the macro mineral composition of ostrich seminal plasma. Sperm concentrations were obtained by use of a spectrophotometer (Spectrawave, WPA, S800, Biochrom) in 20 μl semen diluted 1:400 (v/v) with a phosphate buffered saline solution containing 10% formalin. The transmittance values of the spectrophotometer were used to calculate sperm concentration using a regression equation pre-experimentally developed using the actual sperm counts from a haemocytometer for the ostrich. Neat and diluted samples were evaluated for sperm specific functions that included sperm cell motility, viability and membrane integrity.

2.3. Sperm function evaluation

2.3.1. Sperm cell motility

Sperm images were captured using the Sperm Class Analyzer® (SCA) version 5.3 (Microptic S.L., Barcelona, Spain) with a Basler A312fc digital camera (Basler AG, Ahrensburg, Germany), mounted on an Olympus BX41 microscope (Olympus Optical Co., Tokyo, Japan), equipped with phase contrast optics. All sperm cell motility recordings were made after re-suspension of neat sperm as well as treated sperm in a standard motility buffer using sodium chloride (150 mM) and TES (20 mM) with male specific seminal plasma (2%) to a final sperm concentration of 20×10^6 sperm cells/ml. After re-suspension, the tube was placed in a 38 °C water bath for 1 min. For sperm cell motility recording, 2 μl of diluted semen was