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Introduction

Reproductive technologies such as artificial insemination (AI) and sperm cryopreservation (i.e. freezing) have become high priorities for increasing reproductive success in captive elephants throughout the world. The current populations of Asian and African elephants in North America (managed by the Elephant Species Survival Plan, SSP) are not currently self-sustaining, and are estimated to become nonviable within a few decades unless reproductive rates increase dramatically. Unfortunately, there are only a limited number of breeding bulls and even fewer elephant breeding centres. This has created an urgent need to develop semen cryopreservation techniques and bank samples from all available bulls within the SSP. To reverse these trends and create sustainable populations of healthy elephants, more research is needed to increase our understanding of their basic reproductive biology and optimize breeding management strategies, including the use of assisted reproductive technologies.

Semen collection for artificial insemination

Currently, fresh-extended semen is typically utilized for AI due to the limited success in elephant sperm cryopreservation. Semen samples are collected, extended, cooled and transported within 12 hours to the AI facility for several consecutive days coordinated to the female's estimated time of ovulation. Due to the uncertainty of obtaining semen samples with acceptable quality for AI, utilizing fresh-extended semen requires the recruitment of multiple bull holding facilities to collect semen. However, even the safeguard of recruiting multiple bulls for collection is highly unpredictable, at times leaving AI institutions with either no or suboptimal semen quality after shipment. As a result, investigations to improve sperm storage and develop cryopreservation techniques in elephants have become major areas of focus.

Semen extender and short-term storage

Investigations to improve storage conditions for fresh-extended semen for both Asian and African elephants are necessary to enhance AI efficiency and to advance techniques for cryopreservation. Currently, studies are being performed to formulate the optimal extender (i.e. diluent) and storage temperature that will protect and extend the viability and longevity of Asian elephant spermatozoa during storage. Improvements in semen preservation have far reaching potential. In addition to directly enhancing AI efficacy and providing fundamental information towards improving cryopreservation methods, improved semen preservation also allows additional studies to be performed that were previously unachievable due to reduced sperm survival after short-term transport (i.e. < 24-48 h).

Sperm sex sorting

Studies to develop successful sperm sex sorting techniques in both Asian and African elephants are currently being investigated. Spermatozoa sorted for the female X-chromosome may be utilized for AI to increase the probability of adding younger females to the captive population. The goal is to establish an exponentially growing captive elephant population by creating a gender-skewed captive elephant demographic with a higher

ratio of younger and reproductively viable females to males. Sperm sex sorting requires fresh and viable semen samples to be shipped to a geographically central location due to the specialized equipment required for this procedure. Although the differentiation between X- and Y-chromosome bearing sperm has been successful in both Asian and African elephant spermatozoa, the consistency and availability of obtaining high quality samples (i.e. high motility and viability) after transport has been a major setback, reducing the potential of sperm sex sorting. For this reason, studies to optimize spermatozoa longevity and viability of fresh-extended semen after short-term transport are a priority to further advance elephant reproductive technologies.

Sperm cryopreservation

The advantages of improved sperm cryopreservation are numerous: i) ensure the consistent availability of good quality semen for AI; ii) reduce the cost and logistics associated with using cooled fresh-extended semen; iii) encourage species conservation and preservation by facilitating the development of a genome resource bank; iv) optimize genetic diversity and captive population management; and v) help maintain a healthy elephant meta-population.

Cryopreservation of Asian elephant sperm has posed many challenges, with many samples exhibiting low sperm survival and viability after thawing. By contrast, cryopreserved African elephant sperm has had a relatively higher post-thaw sperm survival compared to Asian elephants, and has resulted in at least one successful pregnancy following AI. Although the exact cause of this discrepancy remains unknown, one possible explanation has been attributed to the species-specific differences in sperm membrane composition. The acrosome, an enzyme-filled sac located at the anterior portion of the sperm head, also appears to be more sensitive in Asian elephants. Since the acrosome plays a key role in sperm-oocyte binding and oocyte penetration during fertilization, a prematurely reacted or damaged acrosome can potentially compromise the fertilizing capability of spermatozoa (Figs. 1 & 2). As a result, species-specific differences in membrane composition and/or structure may influence the physical properties of a membrane, determining how well a sperm cell survives through temperature induced membrane transitions during cryopreservation.

Seminal plasma analysis

Biochemical and molecular differences in semen between the two elephant species may also be a factor influencing these cryo-protective differences. In fact, one of the major challenges in developing successful semen cryopreservation for Asian and African elephants is the high variability frequently observed in semen quality after manual collection. Interestingly, ejaculate quality from the same bull can vary from containing >70% motile sperm to 0% motile sperm in subsequent collections. The underlying cause(s) of this remains unknown, since many of the bulls exhibiting this phenomenon are proven breeders through natural breeding. Because spermatozoa are mixed with accessory gland fluids that provide both nutrients and other factors, individual differences in these components of the semen can influence the overall semen quality. Investigations to analyze molecular and biochemical differences in ejaculates exhibiting varying semen quality are underway, while comparing individual, intra- and inter-species differences. Alternative methods of semen collection that may produce less variable ejaculates, while remaining safe and non-invasive for the individual bull and elephant handlers are also being investigated.

Conclusions

Increasing our knowledge of basic elephant semen biology and sperm preservation will help enhance and ultimately increase reproductive rates of both Asian and African captive elephant populations. This information and associated reproductive technique development may also one day be important to the genetic management of in situ elephant populations, or at the very least provide genetic material through genome resource banks that can serve as a hedge against extinction.

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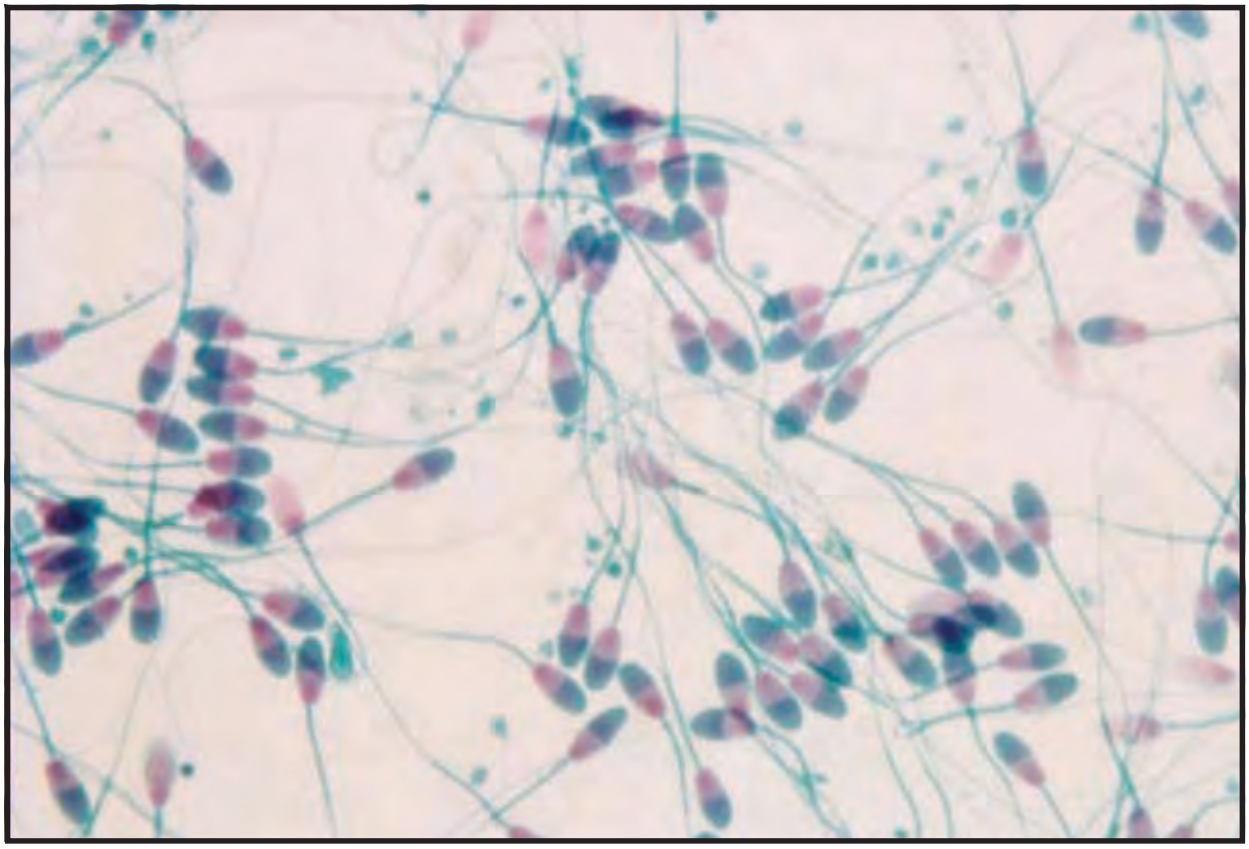
China

When they came out of the forest, the elephants stepping into paddy fields happened to "catch"



Asian elephant spermatozoa with Spermac™ stain to evaluate acrosome status