A NEWLY DEVELOPED ARTIFICIAL INSEMINATION TECHNIQUE IN AFRICAN RHINOCEROSES

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Abstract

Successful captive African rhinoceros breeding management is a priority among zoo and wildlife organizations worldwide. Captive populations have been maintained by collecting individuals from the wild, because of the unsatisfactory reproductive success of animals in captivity. Conservation and safety concerns, along with the growing acknowledgment among rhinoceros caretakers that removing females from their familiar environments for breeding loans can cause distress, have all contributed to the need for the development of assisted reproductive techniques. Demonstration of a successful technique for artificial insemination (AI) would open up many possibilities for captive rhinoceros management, including the collection of genetic material from the wild for integration into captive populations, once semen cryopreservation techniques have been perfected.

A newly developed reproductive strategy involving the application of ultrasonography for reproductive assessment and non-surgical AI has been implemented in the rhinoceros management program at the 15 European and North American zoos over the last 2 yr. The ultrasound assessment, semen collection and AI technology for this rhino project were developed at the Institute for Zoo and Wildlife Research in Berlin. The technique for transrectal ultrasonography in rhinoceroses is performed in standing or laying position with or without the use of anesthetics and restrictive devices. Feces are removed manually with the use of ultrasound gel for lubrication. The rectum is then irrigated with lukewarm water. A battery driven, real-time, B-mode ultrasound scanning system (Sonsite 180) was mainly used. For visualizing the caudal component of the urogenital tract (vestibule, urethra, vagina, urinary bladder, cervix, caudal corpus uteri) a 4-2 MHz transducer was manually introduced into the rectum with ultrasound gel for coupling. To visualize the cranial component of the genital tract (cranial corpus uteri, uterine horns, ovaries, surrounding tissues) a 4-2 MHz transducer or 4-9 were attached to a specially customized probe extension (German patent) and guided manually into the rectum.

Ultrasonography provided valuable information on ovarian activity, uterine integrity and reproductive disease or dysfunction. It was used to visualize structures of the entire reproductive tract. Specifically, it could detect evidence of endometrial cystic degeneration, tumor growth, and indicate the gestational capacity of the uterus. Ultrasound was also useful to detect pathologic changes in the oviduct and its neighbored connective tissue (paraovarian cysts). The ovaries were also screened for pathologic structures such as cysts and atrophic processes of the parenchyma.

Coupled with endocrine information from fecal samples, continuous ultrasonography can help predict the day of ovulation and thereby greatly enhance the possibility for successful AI.

The AI component of this project involved simultaneous imaging by ultrasonography and endoscopy for verifiable semen placement. The insemination technique (submitted as German patent) is non-surgical and has resulted in verifiable sperm deposition directly into the cervix and/or uterus. Ultrasound-guided AI has been attempted, to date, in one black and eight Southern white rhinoceroses in a total number of 11 trials. The nine AI candidates (age ranged from 10-29 yr) have been monitored for sexual cycle activity based on progesterone metabolite levels in the feces at least a half year prior to the AI. The ovulation had to be hormonally induced in six of the nine AI candidates, because of the absence of any cyclic activity.

Semen was collected by electroejaculation placing a customized stimulation probe with a diameter of 10 cm inside the rectum directly above the accessory glands. The electric stimulations were combined with intensive massages of the pelvic and penile urethra. A maximum of 20 stimulations were administered in a duration of about 15 sec, at a maximal voltage of 15 V and 800 milliampere during the electroejaculation. The procedure took on average about 20 min. A total of 30 semen samples were successfully collected from 20 male white rhinoceroses, including two Northern white rhinoceros. In addition, semen was also successfully collected in one black rhinoceros during two electro-stimulations. Ejaculate volume, concentration and pH, and sperm motility and viability were assessed at collection. The samples were fractionated, and each fraction was assessed separately before combining. The semen was diluted 1 to 1 with a special rhino semen extender (BC 1) before shipment as fresh semen or cryopreservation. Extended semen was transported by air at 4°C in a refrigerated vessel. Preliminary trials had revealed that the extended semen maintained good motility for 1-2 days after collection. The sample was warmed to 37°C prior to insemination. Samples used for AI trials ranged in motility status from 60-95%. The AI candidates were anesthetized and positioned in lateral recumbency. Aseptic technique was employed throughout the procedures. First, a flexible speculum was inserted into the vestibule to slightly distend the reproductive tract for optimal endoscopic visualization and a guide wire was placed into the vagina by passing it through the 2 mm wide hymenal opening in nulliparous females. Following this guide wire allows the blind positioning of the insemination catheter in the vagina. By transrectal guidance this catheter was manipulated through the cervical rings so that finally the tip of the catheter was placed in the cranial part of the cervix or caudal part of the uterus. The actual introduction of semen was monitored sonographically to verify its placement.

None of the 11 inseminations performed were successful. Summarizing our previous results, the major obstacle in rhino AI is the timing of the procedure in relation to the natural or induced ovulation in the female AI candidate. Further collaborative research is needed to overcome this problem so that we hope to report in the near future about the first successful AI in an African rhinoceros species. This project has been a team effort, requiring the cooperation and expertise of many individuals: curators, keepers, researchers, pathologists, volunteers, educators and public relations specialists.

PROCEEDINGS

AMERICAN ASSOCIATION OF ZOO VETERINARIANS

ANNUAL CONFERENCE

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