

Reproductive assessment and development of artificial insemination in the white rhinoceros (*Ceratotherium simum simum*)

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A rapidly aging captive southern white rhinoceros (*Ceratotherium simum simum*) population and problems associated with the low rate of reproduction are of great concern (San Diego Workshop, 1998). Increased evaluation of the reproductive soundness of male and female white rhinoceroses and the development of assisted reproduction techniques may help to understand and overcome long standing difficulties in breeding rhinoceros in captivity. Ultrasonographic assessment of the reproductive status of zoo animals has become a widely established technique which, using suitable modifications, is feasible in all rhinoceros species. This technique has proven to be an efficient tool for reproductive health assessment and for selecting potential candidates for natural or artificial breeding in other species. Different phases and aspects in the development of AI in the white rhinoceros are described and their potential implication for natural breeding efforts is assessed.

Long-term endocrinological monitoring gives invaluable information on the cyclicity, erratic cyclicity or acyclicity of breeding aged females. In order to evaluate the health status of the reproductive tract, not accessible by endocrinological methods, and to select potential AI candidates, a transrectal ultrasonographic examination was performed in 29 females aged 4 – 30 years. Animals were either examined lateral recumbent or examined in standing position in a restraint chute. Different forms of progressive ovarian and uterine pathologies were found. Independent from whether females were cycling or acyclic, single up to hundreds of uterine cysts, uterine fluid accumulation, chronic endometritis, uterine leiomyoma and adenoma and para-ovarian tumors were imaged. Causes for these alterations might be based on individual disposition or age related. Due to the advanced status of the alterations, 8 females were considered post reproductive. The females considered post reproductive were ≥ 27 years. Females free of genital tract alterations were aged ≤ 12 years.

In females with no estrous cycle different sonographic types of these so called "flatliners" were characterized. The type I flatliner was characterized by small inactive ovaries with no functional structures, type II by persistent luteal structures, type III by ovarian cysts and type IV found in subadults with constant follicular development. The sonographic identification of the cause of flatlining appeared as an essential prerequisite for a potential hormonal intervention and the successful attempt of an estrous cycle induction.

Electroejaculation was applied in 14 males to evaluate the reproductive condition in immobilized, mature breeding bulls. Imaging of the sex glands prior to semen collection provided information on the development, status and location of the accessory sex glands for precise positioning of the electrostimulation probe. A stimulation probe designed for megavertebrates was rectally inserted and manually positioned on the accessory sex glands. A control panel regulated current and voltage of each applied stimulus and recorded mucosal temperature during the ~5 min of total stimulation time. 3-7 ejaculatory fractions were collected with a maximum volume of 5 - 300 ml. The reproductive assessment categorized males in satisfactory, questionable and unsatisfactory potential breeders according to spermatological results. The cause of reduced or poor reproductive condition in 9 males

remained speculative but management changes, translocation or introduction to unfamiliar breeding aged females may have positive influence on the current breeding status of each potential breeder.

The reproductive assessment of potential female and male breeders by means of endocrinology, ultrasonography and electroejaculation was a prerequisite for the selection of suitable AI candidates with a sound reproductive tract and good reproductive condition. In three flatlining females estrous induction protocols based on the long term application of synthetic progestins and hcG were applied to obtain the exact timing for AI. In two females AI was timed according to behavioral signs and sonographic findings of estrous.

The two main anatomical obstacles for assisted reproduction in the female rhinoceros were the hymenal structure in nulliparous animals and the dense and tortuous cervix. Video-chip-endoscopy, ultrasonography and customized insemination catheters were simultaneously used to deposit the fresh semen deep inside the female's genital tract. Ultrasonography verified the intra-cervical or intra-uterine position of the insemination catheter. Two semen donors had been electroejaculated to ensure a semen sample on the day of AI. The semen samples had been assessed, extended and transported between collaborating institutions. So far one female was successfully impregnated but did not maintain the pregnancy.

Reproductive tract ultrasonographic examination and electroejaculation proved decisive as tools for the assessment of the reproductive condition in male and female white rhinoceros. Ultrasonographic and spermatological evaluation of potential breeders paired with endocrinological monitoring yielded essential information for future breeding recommendations. To date, AI attempts in the rhinoceros have been rare because of the complexity of such procedures. Thanks to a collaborative effort of several individuals and institutions combined expertise on endocrine activity, reliable anaesthesia protocols, the evaluation of the reproductive soundness of AI candidates and prediction of ovulation have contributed to first AI attempts in this species. The successful development of an AI technique in one rhinoceros species might be the key to enhance conservation efforts in other rhinoceros species in future, increasing the genetic diversity and breeding success in captive and wild populations.

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