ALL PHOTOS COURTESY OF IZW BERLIN

Breeding Rhinos for the future

The Artificial way

BY FELIX PATTON

Captive breeding programmes have had some spectacular successes in saving species and reintroducing them into the wild. All rhino species are at risk from poaching, disease and natural disaster. Developing captive populations is an essential safeguard to avoid extinction but breeding success has been limited. If a female rhino does not get pregnant, her uterus starts to develop irreversible problems, such as cysts and tumours. Can the failure to become pregnant be overcome?

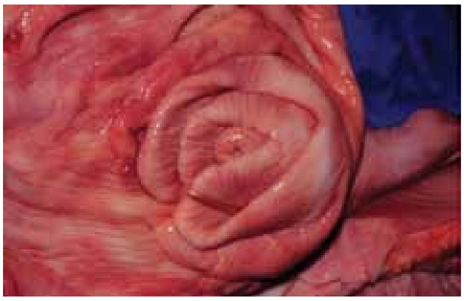
310
Number of oestrous cycles non-reproducing White rhino female in captivity can exhibit.

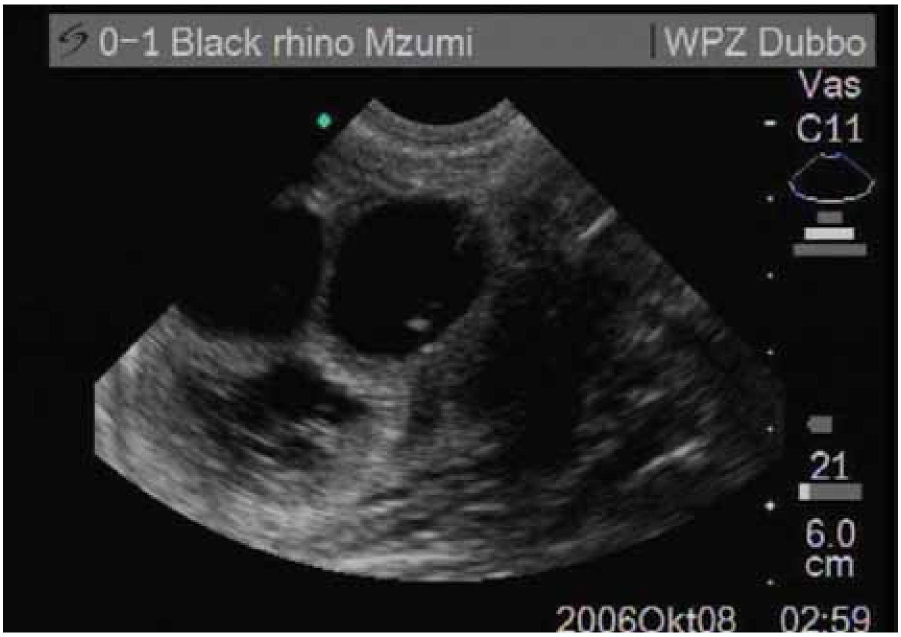
To date, captive reproduction in the White rhino has been poor. Despite the wealth of knowledge acquired over many decades about the husbandry and reproductive needs of captive Black and Indian rhinos, when applied to White rhinos, it has been unsuccessful. The main problem has been the absence of or erratic nature of oestrous cycle activity in over half of the females in the European and North American Species Survival Program.

White rhino females in the wild usually experience short intervals between successive births, even as little as 18 months. This indicates that pregnancy and lactation are the most common endocrine profile with possibly as few as 30 oestrous cycles per reproductive life span.

A reproducing female White rhino in captivity may produce up to nine calves. With a pregnancy of 16 months and subsequent lactation of approximately 12 months, the female exhibits only 90

Ultrasound technology for use in rhinoceros has been used in well over 150 reproductive assessments in more than 70 rhinos with the result that the causes of poor captive performance are now known.





oestrous cycles during her reproductive life span. With pregnancy and lactation dominating the endocrine status, the oestrous cycle is a rare event during her reproductive life.

A non-reproducing White rhino female in captivity exhibits as many as 310 oestrous cycles. They display 90 oestrous cycles by the age of 16 years. These non-reproductive periods of 10 – 15 years in female rhinos were not previously considered problematic. Now it has been found that 16 years old is the same age at which the first signs of disease-caused alterations are detected in the genital tract.

Ultrasound technology for use in rhinoceros has been used in well over 150 reproductive assessments in more than 70 rhinos with the result that the causes of poor captive performance are now known. Female captive rhinos can develop uterine tumours, endometrial cysts and ovarian cysts. These conditions occur more often as the animals age and when they have not achieved a pregnancy. This 'asymmetric ageing process' of the reproductive organs can be prevented with the achievement of at least one pregnancy whether natural or by artificial means. Reproductive problems are relatively non-existent in the wild since females are either pregnant or lactating during the greater part of their reproductive life. This means that regular assessment of reproductive fitness and early breeding are essential.

TOP LEFT: Checking a rhino pregnancy using ultrasound.

TOP LEFT: Portion of a rhino cervix showing folds.

TOP RIGHT: Ultrasound image from a black rhino in Australia.

BELOW: Opened cervix with probe through the convoluted cervical canal.

9

Number of calves a reproducing female white rhino can produce in captivity.

With males, it has been found that unsuitable social systems impact reproductive performance, resulting in half the captive population being sub-fertile or infertile.

Well-established solutions to female infertility in domestic species and humans include artificial insemination (AI), the in vitro production of embryos by means of in vitro maturation (IVM), gamete intrafallopian transfer (GIFT), in vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI), and subsequent embryo transfer (ET) – together known as assisted reproduction technologies (ARTs). Development of these technologies for rhinos was initially slowed by the complicated structure of the reproductive organs. The main challenge to AI was to develop a special catheter to pass through the convoluted passage in the female's cervix to deliver the sperm to the uterus.

The cervix is very firm and strongly folded. This twisty route is the reason male rhinos spend about an hour and a half having sex because they must try to fill up the cervix with sperm.

For egg collection, new equipment and concepts had to be developed to get to the ovary, some 1.5 metres inside the animal (compared to the 15 centimetres to a human ovary). A hollow needle, more than one metre long, fastened to an ultrasonic head and inserted through the rectum is used.

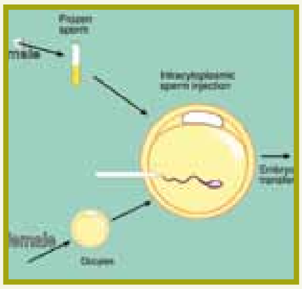
The needle can be viewed on an ultrasonic screen so the ovaries can be seen, enabling the intestine to be accurately punctured, the needle pushed towards the ovaries and the egg cells sucked in. The method can reliably yield many egg cells.

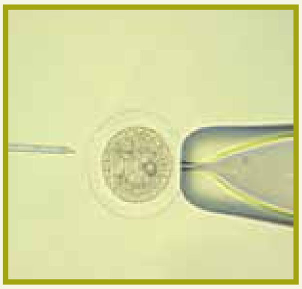
FELIX PATTON is a rhino ecologist writing and broadcasting about the species from Africa and Europe.

Frozen sperm storage.

ARTIFICIAL INSEMINATION (AI)

Non-surgical AI in White rhinos has been achieved using fresh or cryopreserved semen. Additionally, rhino sperm has been successfully sorted into high-purity X and Y chromosome–bearing populations. Critically small, captive rhino populations could be boosted by using only X chromosome-bearing sperm, introduced by AI, to produce female offspring, enabling accelerated population growth.

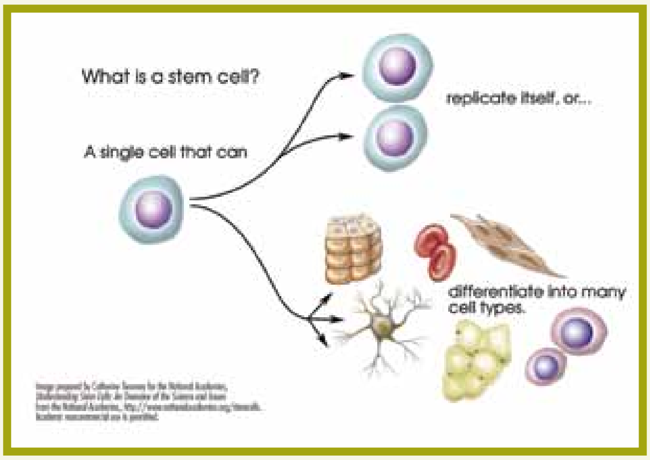




IVF, ICSI, EMBRYO TRANSFER

In domestic species and humans, IVF (in vitro fertilisation), ICSI (intracytoplasmic sperm injection) and ET (embryo transfer) are well- established techniques. Where infertile, female rhinos have a history of reproductive lesions, egg cell collection and in vitro ARTs represent the only option to preserve the female genetic material and to contribute to the diversity of a population.

For example, the Northern White rhino (*Ceratotherium simum cottoni*) is now believed extinct in the wild. Only eight remained in captivity and the females had mostly become infertile so could not contribute to the genetic pool. Two males and two females were recently moved to an enclosed area in Kenya in the hope that they might breed in a more open environment (see SWARA 2010:01). An alternative/additional approach could be to use ARTs to obtain eggs from any of the living females, fertilise with male sperm in the laboratory and implant resulting embryos in either Northern females or, as is more likely, Southern White females (*Ceratotherium simum simum*) as surrogate mothers. Current rhino embryo production techniques require refinement for consistent success.



THE CHIMERA APPROACH

The body is made up of many hundreds of different types of cells, all of which come from a pool of stem cells in the early embryo. Various types of stem cells give rise to the cells that carry out the specific functions of the body, such as skin, blood, muscle, and nerve cells.

There are 3 types of stem cell that can be used, each with limitations:

i) **Embryonic stem cells** are a primitive type of cell that can be coaxed into developing into all of the types of cells (e.g. blood/heart/brain /nerve cells, etc). In the past, they have always been derived from embryos in a process that causes the latter's death, and this has been considered unethical.

ii) **Adult stem cells** bear some similarities to embryonic stem cells but are limited in flexibility, and are only capable of developing into a few of the cell types.

iii) **Induced pluripotent stem cells** are ordinary cells such as skin cells that are specially processed to exhibit some of the properties of embryonic stem cells without the ethical and rejection problems.

Japanese scientists have developed a method to turn adult cells, such as skin cells, into embryonic-like cells. In the future, it may (or may not) be possible to use the method to create embryonic cells from a Northern White rhino and blend them with the embryos of the Southern White rhino. The resulting embryo is a "chimera", with a mixture of cells from both. Then the hope would be that some of the resulting offspring, grown in surrogate Southern White rhino mothers, would grow up to produce the sperm and eggs of the Northern White rhino.

Scientists at Stanford University in California, USA have grown human eggs and sperm in the laboratory. The team used stem cells taken from embryos but are hoping to use skin cells in future. Once the method is proven it could be refined for rhinos as could other such scientific advances. These approaches would also permit the use of frozen cell samples from dead individuals.

MILESTONES IN THE USE OF ARTs IN RHINOS


Probe used for electroejaculation.

2004

First conception from AI performed in 2004
Artificial insemination was first successfully used to conceive a rhinoceros at Budapest Zoo, Hungary, in 2004.

2005

First birth from AI performed in 2005
The world's first rhinoceros birth from artificial insemination occurred at Budapest Zoo in January 2007.

2006

First Egg Cell Harvest in 2006
Scientists from the IZW, Berlin together with veterinarians from Australia successfully collected egg cells from an infertile, Black rhino. These were then matured in a test-tube and fertilised with rhino sperm but did not grow into embryos.

2008

First IVF Black Rhino Embryo in 2008
In June 2008, researchers successfully created a Black rhino embryo which was cryopreserved until the technology is developed to transfer it to an appropriate recipient.

2008

First birth from use of frozen sperm in 2008
In June 2007, scientists from the IZW, Berlin artificially inseminated the mother with cryopreserved sperm, which had been frozen for three years at minus 196 °C (minus 321 °F) in liquid nitrogen. The world's first White rhino to be conceived using frozen sperm was born in October 2008 at Budapest Zoo. The successful use of frozen semen will enable reproduction experts to anaesthetise wild bulls, collect semen from them, and use the frozen sperm for breeding offspring in international zoos.

2009

AI White rhino born in UK in 2009
The first White rhino to be born in Britain through AI arrived at Colchester Zoo using the pioneering AI treatment developed at the IZW in Berlin.


Standing sedation for fertility treatment.

2010

AI Indian rhino due in 2010
Indian rhino female artificially inseminated in June 2009 using sperm collected four years previously and cryo-preserved at Cincinnati Zoo's CREW CryoBioBank before being thawed and utilized, resulted in the birth of a male calf in October 2010. This shows that it is possible to produce offspring from behaviourally incompatible Indian rhino pairs and allow new genetic material to be introduced in captive populations globally.

Collecting and banking sperm from genetically valuable male rhinos is an important step toward prolonging the genetic life of founder animals and preserving the genetic potential of males that may otherwise never contribute to the captive population.

ARTs for rhinos have been successful but the technologies need further refinement to obtain greater consistency. With appropriate funding the timescale should be short and a lifeline secured for those rhino populations heading rapidly towards extinction.

GLOSSARY

Artificial Insemination – the introduction of sperm into the female by means other than sexual intercourse.
Catheter - A hollow, flexible tube for insertion into a body cavity, duct, or vessel to allow the passage of fluids or distend a passageway.
Chimera - an animal that has two or more different populations of genetically distinct cells that originated in different fertilised eggs.
Chromosome - components in a cell that contain genetic information.
Cryopreservation - to preserve biological tissue by freezing it and holding it at very low temperatures.
Embryo Transfer - placing an egg fertilised outside the womb into the female's uterus or Fallopian tube.
Endocrine – secretion of chemicals directly into the blood stream.
Endometrial – pertaining to the endometrium, which is the lining of the uterus.
Gamete Intrafallopian Transfer - a technique in which an egg is fertilised outside the body, then implanted into one of a female's Fallopian tubes.
Intracytoplasmic Sperm Injection - a technique whereby a single sperm can be injected directly into an egg in an attempt to achieve fertilisation.
In vitro Fertilisation - a process by which egg cells are fertilised by sperm outside the womb.
In vitro Maturation – where eggs cells are matured in the laboratory rather than the mother's body.
Lactation – the period following birth during which milk is secreted.
Oestrous Cycle – regular repeated stages by which the body of a rhino female is prepared for reproduction.
Ultrasound – high frequency sound waves whose echoes can be turned into a picture.
X chromosome – the sex chromosome associated with female characteristics.
Y chromosome – the sex chromosome associated with male characteristics.