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## THE SABAH RHINO BREEDING PROGRAMME: CURRENT SITUATION AND FIRST CONCLUSIONS

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The Sumatran rhinoceros (*Dicerorhinus sumatrensis*) is on the verge of extinction. Once found throughout Southeast Asia, it stands now with less than 100 individuals scattered mainly in three national parks in Sumatra. In Sabah (Malaysian Borneo), as well as in Peninsular Malaysia, the Sumatran rhinoceros is now considered to be "functionally extinct". Between 1984 and 2014, 45 Sumatran rhinoceroses were captured from the wild, resulting in only two successful breeding pairs. In early 2015 only nine individuals survive in captivity, three (1.2) in the Borneo Rhino Sanctuary, in Sabah; five (2.3) in the Sumatran Rhino Sanctuary, in Sumatra; and only one (1.0) left in Cincinnati Zoo, USA. Since 2009 the Leibniz Institute for Zoo and Wildlife Research (IZW) has been collaborating with the Borneo Rhino Alliance (BORA), mainly through the use of advanced imaging and assisted reproduction technologies on the 1.3 wild-caught Bornean rhinoceroses (*Dicerorhinus sumatrensis harrissoni*) held at the Borneo Rhino Sanctuary. Throughout 14 visits to Sabah, the IZW team and associated experts had the chance to perform 42 reproductive assessments, six semen collections by electroejaculation, three endometrial cyst removal procedures, one artificial insemination (AI), three oocyte collections ("ovum pick-up", OPU) and one intracytoplasmic sperm injection (ICSI). In what concerns the male, significant improvements in semen quality were observed throughout time: despite being considered reproductively inactive in 2009 and 2011, in 2014 a maximum concentration of  $5 \times 10^6$  sperm cells /ml was obtained, with 65 % motility. When captured from the wild, two cycling females showed severe reproductive pathology that rendered them incapable of carrying a pregnancy, namely extensive cystic endometrial hyperplasia and a large number of uterine leiomyomas. Four different techniques were used to remove endometrial cysts: uterine lavage with cell medium M199 (Sigma-Aldrich Chemie GmbH, Munich, Germany) and povidone-iodine solution (Braunol<sup>®</sup>, B. Braun Melsungen AG, Germany), endoscopic assisted laser photoablation and ultrasound guided aspiration. These procedures proved to be of very limited success. Consequently, AI was attempted only once and no fertilisation occurred due to poor condition of the uterus and irregular cycling. As natural conception was excluded, *in vitro* fertilisation was attempted for the first time in Sumatran rhinoceros. OPU was performed on three occasions and successful on two, with the total collection of five oocytes. ICSI was performed with two of the oocytes, but cleavage was not achieved. Furthermore, to preserve the genome of these that may well be the last Bornean rhinoceroses, cell cultures were established from skin and mucosal samples and cryopreserved until seven passages, for future development of induced pluripotent stem cells.