

Birth of a Siberian Tiger Cub from an Albino Mother Tiger with Help of eCG and hCG

Yoon-jeong Choo, Myung-soo Park, Hyo-dong Han, Gye-sun Ham, Young-sun Park, Gyeong-sik Kim, Sun-duk Park, Yang-mook Lim, So-young Jung and Hwanyul Yong*

Seoul Zoo, Gwacheon 427-080, Korea

ABSTRACT

This is about the successful use of eCG and hCG for producing a Siberian tiger pup born from 10-year-old, primiparous, albino Siberian tiger. From February 2010 to July 2010, natural breeding had been tried three times with no conception. During this period of five months, estrus behaviors appeared to be typically normal and a lot of matings were observed. After consecutive failures, 1000 IU eCG (equine chorionic gonadotropin) were intramuscularly injected on the day showing estrus behavior, followed with an injection of 750 IU hCG (human chorionic gonadotropin) 80 hours later. The tiger stopped recurrence of estrus, and a cub, weighed 780 gram, was born alive 104 days after hCG injection. This study is the first report showing the unique, successful use of exogenous hormones as one of artificial breeding programs in the long history of captive breeding of carnivorous zoo animals in Korea.

(Key words : Siberian tiger, albino tiger, eCG, hCG, cub)

INTRODUCTION

Siberian tiger (*Panthera tigris altaica*) is classified as an “endangered (EN)” status by IUCN (International Union for Conservation of Nature), and also protected by Korean government as “Class I” of national endangered wild animal and botanical resources. Depending totally on natural breeding is not appropriate especially in the situation of domestic zoos where enclosures are small compared to the number of tigers. To prevent inbreeding, various reproductive techniques must be applied to tigers, when those skills can guarantee controlled breeding in the tiny space of tiger enclosure.

We used the byproduct of inbreeding tigers, albino female tiger, to prove the usefulness of exogenous hormones, eCG and hCG, which have been successfully used for artificial breeding of feline carnivores in advanced countries (Donoghue et al., 1993; Howard et al., 1997; Crichton et al., 2003; Pelican et al., 2006). A single cub was born, but primiparous albino mother tiger, genetically defected, failed to care about her daughter tiger. We hope this study would contribute to igniting artificial breeding of zoo carnivores in Korea.

CASE REPORT

The albino female tiger was born at Seoul zoo in September

9, 2000 that has had visual problems such as strabismus from the beginning of birth, assumed to be the byproduct of long-term inbreeding. So far, making more albino white tigers had been tried and produced to run a safari type of exhibition, full of white tigers at a zoo in Korea. Also at Seoul zoo in 2010, natural breeding with a normal Siberian male tiger, born in June 21, 2006, was conducted. Lots of matings were observed but failed to conceive over three consecutive periods. The first encounter the male tiger at the same enclosure was Feb 24 to 27 at which 17 times of mating were randomly observed by zoo keepers. May 4, 66 days later from the last mating, the female showed estrus signs again so that she was rejoined with the same male tiger. Making 25 times of mating were noticed May 7 to 8. However, 60 days later, July 9, she was caught showing estrus signs like decreased appetite, rolling down on the concrete floor, and again rejoined with the same male, matings just observed 3 times. During the trials, estradiol and progesterone were analyzed by Time-Resolved Fluoroimmunoassay using the feces of the female tiger (TR-FIA) (Yong et al., 2009). Even though the data from the analysis of collected feces of the female tiger are not easy to read, we can see matings allowed by the female tiger start showing when estradiol level decreased and progesterone increased. In addition, the recurrence of estrus might be caused by the failure of ovulation determined by the irregular levels of progesterone (Fig. 1).

* Correspondence : E-mail : getzoopregnant@seoul.go.kr

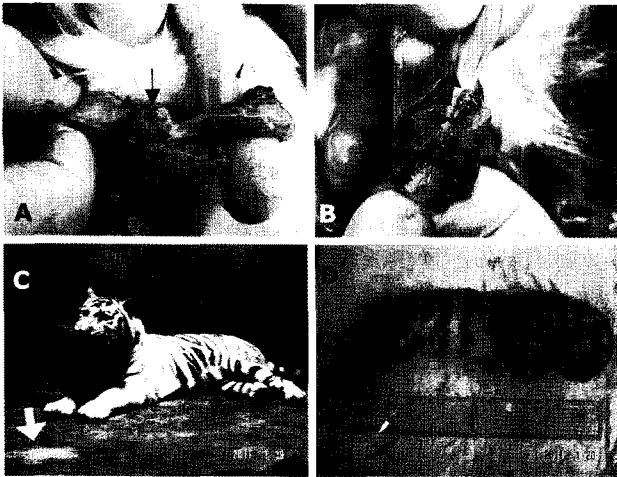


Fig. 1. Domestic cat ovaries 4 days after eCG and hCG injections (A and B), and an albino mother tiger and her dead female cub (C and D). (A) On the surface of left ovary is shown 4 corpus lutea (arrows). (B) Five corpus lutea (arrows) is observed on the surface of right ovary. (C) The albino mother tiger rests on the concrete floor of indoor enclosure 5 days after parturition. The arrow indicates white-colored, mucosal lochia, meaning uterine involution was normally processed. (D) The appearance of a single cub, 780 gram, less than normal weight, was much closer to normal fur color of Siberian tiger.

Early in the morning of Sep 29, 2010, the female tiger refused to eat chicken meat, rolling over on the concrete floor. Finally, 1000 IU of eCG (Folligon[®], Intervet; Netherlands) was intramuscularly injected using blowing dart at 2 PM of same day. Eighty hours later, 750 IU of hCG (HCG[®], Daesung Microbiological Labs; Korea) was injected in the same way. The birth of a single female cub was born alive on the night time of Jan 14, 2011, 104 days after the injection of hCG. Zoo keepers found the cub dead inside indoor enclosure the next day.

DISCUSSION

Tiger is non-seasonal breeder and one of animal species in captivity showing no infertility (Donoghue et al., 1993; Donoghue et al., 1996; Crichton et al., 2003). The low litter size shown in this study might be caused by inaccurate volume of eCG injected twice commonly resulted from using a blow dart and not injecting PGF₂α when eCG was injected (Pelican et al., 2006). We used 1000 IU of eCG and 750 IU of hCG in this study (Howard, 1999). According to the species of carni-

vores, ovarian responsiveness to exogenous gonadotropin treatments is so different that the doses of gonadotropins and duration between two injections should be selectively applied (Howard et al., 1997; Tsutsui et al., 2000; Swanson and Brown, 2004; Pelican et al., 2006). This dose-dependent difference is not related with body size of an animal but with the purposes of oocyte aspiration and normal follicular development (Howard et al., 1997; Pelican et al., 2006). In our preliminary study with stray cats, 400 IU of PMSG and 200 IU of hCG was considered to be optimal to superovulate and aspirate oocytes as supportive data for further investigation of ovarian responsiveness in zoo felines (Fig. 1A and 1B) (Lee et al., 2004).

Natural breeding had been tried 3 times before deciding to use gonadotropins in this study. Interesting observations during the consecutive trials of natural breeding are two things, the duration that needed to show estrus behaviors again and the number of matings caught by zoo keepers compared to the mating frequency observed when gonadotropins were used. Sixty to sixty six days was needed for the albino Siberian tiger to show estrus behaviors such as decreased appetite and rolling down on the floor of indoor enclosure. Tiger is induced ovulator. The fact that a lot of matings did not contribute to pregnancy in 3 times of natural breeding and just one trial of exogenous gonadotropins resulted in the production of a cub, meaning most of female captive feline carnivores would be vulnerable to maintaining reproductive soundness (Crosier et al., 2011).

The levels of estradiol and progesterone shown in the consecutive trials of natural breeding were irregular, and the progesterone levels of the second and third natural mating periods, May 7 to 8 and July 11 to 12, do not seem high commonly observed after LH peak (Fig. 2). Lots of matings may not be enough to ovulate.

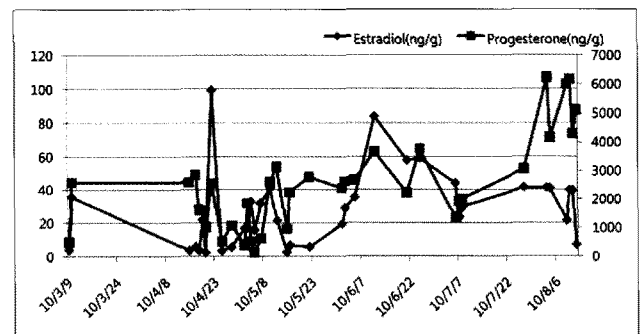


Fig. 2. Changes of estradiol and progesterone observed during several trials of natural mating without help of eCG and hCG.

Nystagmus, lack of stereopsis, and strabismus are clinical signs obviously observed in the albino tiger of this study. Like human albinism, the tiger may have problems of nervous, hematological, respiratory, and gastrointestinal systems as well as integumentary and ophthalmological (Carden et al., 1998). The reason yellow-colored cub was born is that a recessive gene causing albinism must be inherited from both parents (Fig. 1D). Death of a cub after normal parturition was resulted from the immature nursing skill of the mother tiger, but genetically deficits explained above would be in some degree contributive to loss of the cub (Fig. 1C).

In conclusion, this study showed the easy and economical use of exogenous gonadotropins in order to produce tiger cubs for the first time in Korea.

REFERENCES

- Carden SM, Boissy RE, Schoettker PJ and Good WV. 1998. Albinism: Modern molecular diagnosis. *Br. J. Ophthalmol.* 82:189-195.
- Crichton EG, Bedows E, Miller-Lindholm AK, Baldwin DM, Armstrong DL, Graham LH, Ford JJ, Gjørret JO, Hyttel P, Pope CE, Vajta G and Loskutoff NM. 2003. Efficacy of porcine gonadotropins for repeated stimulation of ovarian activity for oocyte retrieval and *in vitro* embryo production and cryopreservation in Siberian tigers (*Panthera tigris altaica*). *Biol. Reprod.* 68:105-113.
- Crosier AE, Comizzoli P, Baker T, Davidson A, Munson L, Howard JG, Marker LL and Wildt DE. 2011. Increasing age influences uterine integrity, but not ovarian function or oocyte quality, in the Cheetah (*Acinonyx jubatus*). *Biol. Reprod.* 85:243-253.
- Donoghue AM, Byers AP, Johnston LA, Armstrong DL and Wildt DE. 1996. Timing of ovulation after gonadotrophin induction and its importance to successful intrauterine insemination in the tiger (*Panthera tigris*). *J. Reprod. Fertil.* 107:53-58.
- Donoghue AM, Johnston LA, Armstrong DL, Simmons LG and Wildt DE. 1993. Birth of a Siberian tiger cub (*Panthera tigris altaica*) following laparoscopic intrauterine artificial insemination. *J. Zoo. Wildl. Med.* 24:185-189.
- Howard JG, Roth TL, Byers AP, Swanson WF and Wildt DE. 1997. Sensitivity to exogenous gonadotropins for ovulation induction and laparoscopic artificial insemination in the cheetah and clouded leopard. *Biol. Reprod.* 56:1059-1068.
- Howard JG. 1999. Assisted reproductive techniques in nondomestic carnivores. In: Fowler ME, Miller RE, editors. *Zoo and Wild Animal Medicine: Current Therapy IV*. Philadelphia PA: WB Saunders Co; pp. 449-457.
- Lee HS, Yin XJ, Lee YH, Min WK, Kim TS, Choi JW, Yoon BC, Kim JI and Kong IK. 2004. *In vitro* maturation of tiger oocytes: A case report. *J. Emb. Trans.* 19:185-189.
- Pelican KM, Wildt DE, Pukazhenthil B and Howard JG. 2006. Ovarian control for assisted reproduction in the domestic cat and wild felids. *Theriogenology* 66:37-48.
- Swanson WF and Brown JL. 2004. International training programs in reproductive sciences for conservation of Latin American felids. *Ani. Rerod. Sci.* 82-83:21-34.
- Tsutsui T, Tanaka A, Takagi Y, Nakagawa K, Fujimoto Y, Murai M, Anzai M and Hori T. 2000. Unilateral intrauterine horn insemination of fresh semen in cats. *J. Vet. Med. Sci.* 62:1241-1245.
- Yong HY, Park SH, Choi MK, Jung SY, Ku DC, Yoo JT, Yoo MJ, Yoo MH, Eo KY, Yeo YG, Kang SK and Kim HY. 2009. Baby giraffe rope-pulled out of mother suffering from dystocia without proper restraint device. *J. Vet. Clin.* 26:113-116.

(접수: 2011. 8. 1 / 심사: 2011. 8. 2 / 채택: 2011. 8. 20)