The Journal of Veterinary Medical Science

NOTE

Wildlife Science

Contraceptive effect of a gonadotropin-releasing hormone vaccine on a captive female African Lion (*Panthera leo*): a case study

Keisuke KAWASE[1,4)#*], Jumpei TOMIYASU[2,3)#], Kazuyuki BAN[1)], Ryosuke ONO[1)], Satoshi ANDO[1)], Aya ONO[1)], Ran KIMURA[1)], Kanako TOMISAWA[1)], Motozumi MATSUI[2)] and Shun-ichi SHIIHARA[1)]

[1)]Omuta City Zoo, 163 Showa-machi, Omuta, Fukuoka 836-0871, Japan
[2)]Obihiro University of Agriculture and Veterinary Medicine, Nishi 2-11, Inada-cho, Obihiro, Hokkaido 080-8555, Japan
[3)]Department of Biodiversity Protection, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn 10-748, Poland
[4)]Present address: Hitachi Kamine Zoo, 5-2-22 Miyatacho, Hitachi, Ibaraki 317-0055, Japan

J. Vet. Med. Sci.
83(9): 1454–1458, 2021
doi: 10.1292/jvms.21-0032

Received: 18 January 2021
Accepted: 6 July 2021
Advanced Epub:
19 July 2021

ABSTRACT. Lions (*Panthera leo*) breed well under captivity, so contraception has been commonly conducted for population management, leading to a demand for a less invasive and reversible contraceptive approach in lions. In this study, we examined the efficacy of a commercial gonadotropin-releasing hormone vaccine as a method of suppressing reproductive activity in a sexually matured female lion. Under behavioral restraint, the vaccine was injected twice (days 0 and 109). After the initial vaccination, ovarian activity is still observed. After the second vaccination, contraceptive effect was confirmed for 246 days until restart of estrous cycles. We confirmed only a slight swelling around the injection site after the second vaccination. This study may suggest an alternative option for a contraceptive method in lions.

KEY WORDS: contraception, gonadotropin-releasing hormone vaccine, husbandry training, immunocontraception

The African lion (*Panthera leo*) is a large feline inhabiting west to Central Africa and is regarded as "vulnerable" as described by the International Union of Conservation of Nature (IUCN) [12], and its population continues to decline, except in intensively managed areas [5]. In contrast, the African lion is a popular species as zoo animals. Because these captive lions have bred well under zoo conditions, their population is likely to exceed available space. Therefore, contraception has been conducted for population management of this captive species [7, 20].

In Japanese zoos, 61% (25/41) of the facilities attempt contraception for lions, mainly through vasectomy [13]. However, due to the size of these animals, the amount of labor needed for surgical procedures is considered a drawback to this method, and anesthesia during surgery causes physical stress to animals. Moreover, permanent sterilization is highly inadvisable for endangered species, considering that gene stock is one of the important functions of zoos. Hence, this suggests the need to consider a less invasive and reversible contraceptive method in lions.

The gonadotropin-releasing hormone (GnRH) vaccine is considered a reversal immunocontraceptive method with few side effects [3]. Vaccination entails the administration of a modified form of the GnRH hormone to stimulate a production of anti-GnRH antibodies, causing the antibodies to bind to the endogenous GnRH and inhibiting the natural binding to their receptors on the pituitary gonadotrophs, resulting in the suppression of gonadotropin secretion [3]. The GnRH vaccine has been a common approach in population management by manipulating female reproductive activity in the field and captive conditions (for instance, cats [16], elephants [8], elks [19], and horses [9]). In this study, we examined the efficacy of a commercial GnRH vaccine as a method of suppressing reproductive activity in female lions. While the female lion is considered as an induced ovulatory animal, captive female lions can ovulate spontaneously and demonstrate an estrous cycle [14, 20]. In our previous study, continuous

*Correspondence to: Kawase, K.: k_2929_k@yahoo.co.jp
#These authors contributed equally to this work.
(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

Fig. 1. The first vaccination to a female lion under behavioral restraint by husbandry training.

samplings under behavioral restraints through husbandry training enable us to clearly monitor the reproductive cycle of a female lion [14]. In the present study, we validated the contraceptive effect using the same monitoring methods.

This study adheres to the Japanese Association of Zoos and Aquariums Ethics and Welfare Guidelines and Caring for Wildlife: The World Zoo and Aquarium Animal Welfare Strategy. A well-experienced veterinarian conducted the procedures in this study, and the animal subject faced no health problems during the experimental period. The subject was a captive sexually mature female lion (93.8 kg) in Omuta City Zoo (Fukuoka, Japan), born in 2011 and gave birth in 2014, and was used in this study from September 2017 to April 2019. The duration of the estrous cycle was 52.6 ± 5.0 days, and the levels of plasma progesterone (P_4) during the follicular phase and luteal phase were 1.5 ± 2.9 ng/ml and 27.2 ± 21.2 ng/ml, respectively [14]. In this study, we used Improvac (Zoetis Japan, Tokyo, Japan), a commercial GnRH vaccine. Improvac was originally designed for pigs for their contraception, with two injections of the vaccine (400 µg) with an interval [2, 10]. Because of the similarity of body weights between lions and pigs, we determined the same injection dose. Under a behavioral restraint using husbandry training, our subject was injected twice subcutaneously (on day 0 and day 109) with 400 µg of the GnRH vaccine (2 ml of Improvac) (Fig. 1). The second vaccination (day 109) was administered by the veterinarian after assessing the contraceptive effect through vaginal smear and observation of the vaginal discharge and evaluating the side effect of the first vaccination.

Reproductive activity of the lion was monitored by measuring the plasma P_4 levels, examining the vaginal smear, and observing a vaginal discharge under the behavioral restraint, as previously described [4, 14]. While the lion was positioned lying on the deck setup along the exhibit fence, 0.5–3 ml of blood was collected from the tail, and vaginal smears were conducted by swab (Hakujiumenbou, Hakujuji Co., Ltd., Tokyo, Japan). The vaginal discharge was evaluated using a ranking scale of 0–2 (0 =absence of discharge; 1 =wet hair around the vagina; 2 =presence of mucous secretion) [14]. Blood sampling, vaginal smear, and observation of the vaginal discharge were conducted twice a week, once every two days, and almost every day, respectively.

The plasma was separated from whole blood by centrifugation and stored at −20°C until assay procedures. To ensure the contraceptive effects after the vaccination procedures, plasma P_4 levels from September 2017 to May 2018 were measured using competitive double-antibody enzyme immunoassays after extraction with diethyl ether, as described previously [14, 18]. The standard curve ranged from 0.05 to 50 ng/ml. Moreover, the intra- and inter-assay coefficients of variation were 4.9% and 2.7%, respectively. To check the vaginal smear, the anuclear superficial cell rate was calculated by microscopy, after staining using a blood smear staining kit (Hemacolor, Merck KGaA, Darmstadt, Germany). In each slide, 200 epithelial cells were counted. We pointed out that P_4 level <5.7 ng/ml indicates that the female lion does not have a functional corpus luteum in reference to the calculated basal level of 5.7 ng/ml from our previous study [14]. Furthermore, during the estrous phase, the proportion of anuclear superficial cells on the vaginal smear was >80% with a vaginal discharge score of 2 [14]. Thus, we also regard them as indicators of estrus.

Adverse effects of the vaccination were evaluated by assessing the injected site and testing blood chemistry. Reactions at the injection site were scored “0” for no visible reaction, “1” for visible swelling, and “2” for visible swelling with lameness, as previously described [9]. We used Fuji Dri-Chem (Fujifilm Co., Tokyo, Japan) for blood chemistry analysis a week prior to the vaccination, and 1 week, 1 month, and 3 months after the first and second vaccinations. We focused on aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and creatinine (Cre) levels as indicators of liver and kidney injury.

After the first vaccination, on day 0, the lion still demonstrated a normal estrous cycle (Fig. 2), suggesting that the initial vaccination did not take effect on the subject. After the second vaccination, on day 109, the following were observed in comparison to the first injection: plasma P_4 concentrations decreased rapidly to basal levels, the proportion of anuclear superficial cells on vaginal smear remained at low levels <80%, and the score of the vaginal discharge was <1 (Fig. 2), altogether indicating that the

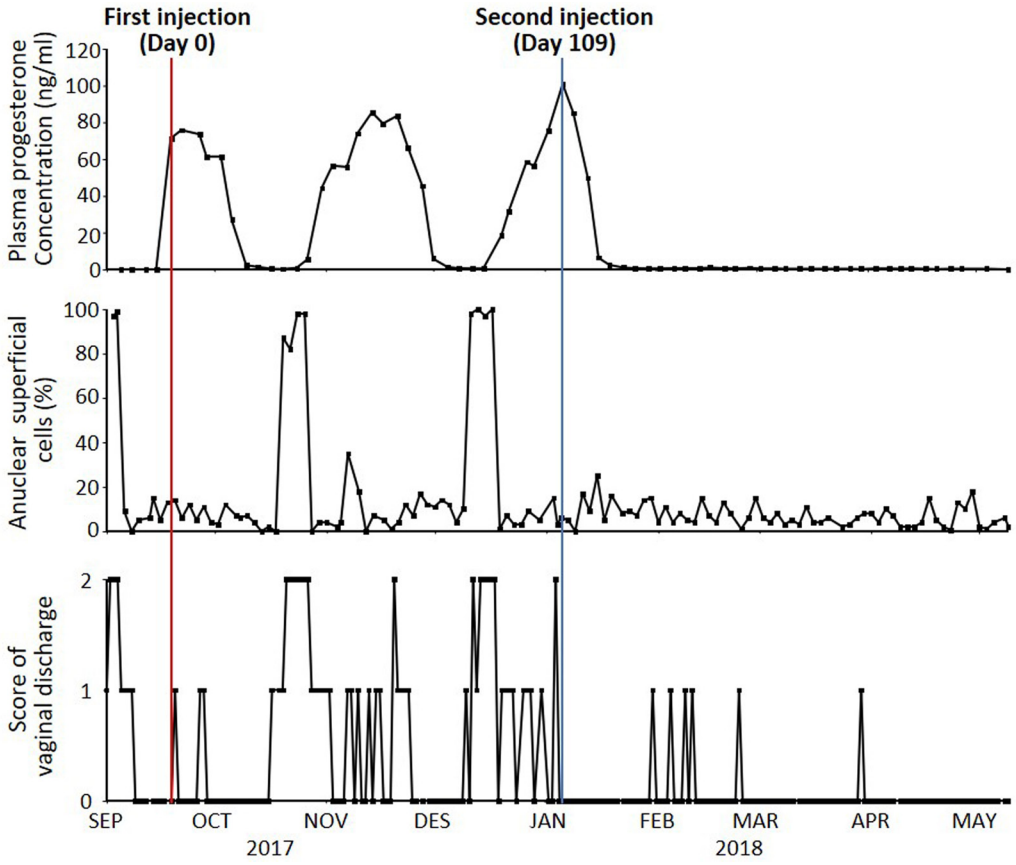

Fig. 2. Contraceptive effects of vaccinations. Profiles in plasma progesterone concentration (ng/ml), proportion of anuclear superficial cells on vaginal smear (%), and score of vaginal discharge (0–2). The red and blue lines indicate the dates of the first and second vaccinations, respectively.

reproductive activity was suppressed. This contraceptive condition continued for 246 days until the first estrus (>80% of anuclear cells and a score of 2 of the vaginal discharge) was confirmed after the second vaccination (Fig. 3).

Furthermore, the injection site showed no reaction (score=0) after the first vaccination; however, a slight swelling (score=1) around the injection site was observed a day after the second vaccination (Fig. 4), although the swelling was not observed the next day. In the previous reports on horses and pigs, temporal swelling and lameness were observed after the continuous vaccinations [9, 10], whereas in other studies conducted on Asian elephant and dog subjects, the adverse effects are never confirmed [1, 17]. Thus, this swelling reaction in the lion is considered a side effect of the second vaccination. There was no difference in values of blood chemistry (AST, GGT, ALT, and Cre) between pre- and post-vaccinations (Supplementary Table 1). In this study, adverse effects of Improvac in the lion were considered minimal. To further confirm this, a larger sample size is required.

After the second vaccination, the vaginal discharge yielded a score from 0 to 1, the proportion of anuclear superficial epithelial cells was <80%, and P_4 concentration remained below the basal level. These results indicate estrus and following luteogenesis did not occur after a second vaccine. Therefore, we confirmed the contraceptive effects of Improvac in the lion. The contraceptive effect of Improvac is dose dependent; for example, in female African elephants, 1,000 µg of Improvac injection is effective, whereas 600 µg may not yield the same level of effectiveness or result [6, 8]. From our results, 400 µg of Improvac was enough for the lion, although this is only confirmed on one animal. In our study, one-time vaccination of 400 µg of Improvac was not enough to suppress the female reproduction in the lion. In contrast, previous related studies revealed that only one injection of 400 µg of Improvac is sufficient in horses and dogs [1, 9]. After a single injection, 85% (47/55) of the mares demonstrated no ovarian activity [9], and testis in the treated male dogs became small in size with no sperm [1]. The results in this study are inconsistent with the aforementioned results, probably due to the difference of vaccination efficiency and/or the injection dose per body weight. While in this study we kept the interval between the two injected vaccines as 108 days to check the contraceptive and side effects of the first Improvac injection, a second vaccination is generally administered within 30 days after the first vaccination [10, 11, 15]. Therefore, in future experiments, considering shorter intervals between vaccinations may be explored. Furthermore, our research revealed that after contraceptive effects continued for 246 days, estrus occurred firstly in the short intervals (12–14 days), and then in longer intervals (45–46 days) as is the same with the duration in the normal reproductive cycle in this female [14]. A previous study demonstrates that mares (n=47/51) restarted reproductive cyclic from 232 to 488 days (median 344 days) after vaccinations [21]. Our results suggest that the contraceptive effect of Improvac is also reversible in lion.

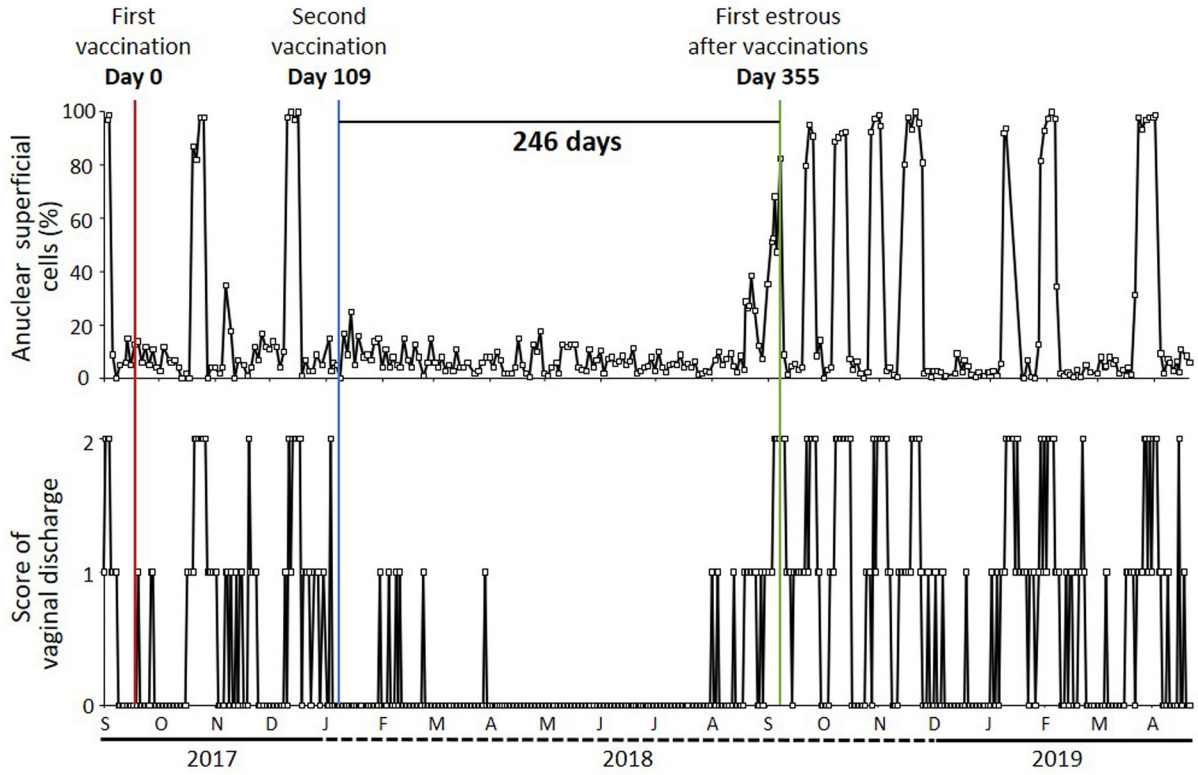

Fig. 3. Reoccurrence of reproductive activity after vaccinations. Proportion of anuclear superficial cells on vaginal smear (%) and score of vaginal discharge (0–2). The red, blue, and green lines indicate the dates of the first vaccination, second vaccination, and first estrus after vaccinations, respectively. Estrus is defined as >80% of anuclear cells and a score of 2 of vaginal discharge based on the previous study (Kawase *et al.* 2018 [14]). Months are abbreviated as the capital letters.

Fig. 4. Slight swelling (dotted line) around the injection site a day after the second vaccination (day 110).

In conclusion, we confirmed the contraceptive method of using Improvac in lions. In Japan, vasectomy is mainly performed to suppress reproduction in lions; however, because immunocontraceptive methods were found to yield fewer side effects and apparent burden to animals, our study may be suggested as an alternative method of using contraceptives that will help promote and improve animal welfare in zoos.

CONFLICT OF INTEREST. The authors have no competing interests to declare.

ACKNOWLEDGMENTS. We thank all the zoo staff for the support of our study.

REFERENCES

1. Ajadi, T. and Oyeyemi, M. 2015. Short-term effects of a single dose of gonadotrophin releasing hormone (GnRH) vaccine on testicular and ejaculate characteristics of dogs. *Bulg. J. Vet. Med.* **18**: 123–131. [CrossRef]
2. Andersson, K., Brunius, C., Zamaratskaia, G. and Lundström, K. 2012. Early vaccination with Improvac®: effects on performance and behaviour of male pigs. *Animal* **6**: 87–95. [Medline] [CrossRef]
3. Asa, C. and Moresco, A. 2019. Fertility control in wildlife: review of current status, including novel and future technologies. pp. 507–543. In: Reproductive Sciences in Animal Conservation, 2nd ed. (Comizzoli, P., Brown, J. L. and Holt W. V. eds.), Springer, Cham.
4. Ban, K., Ono, R., Kawase, K., Saito, A. and Shiihara, S. 2017. Husbandry-training of big cats for blood collection. *J. Jpn. Assoc. Zoo. Aqua.* **59**: 1–6.
5. Bauer, H., Chapron, G., Nowell, K., Henschel, P., Funston, P., Hunter, L. T., Macdonald, D. W. and Packer, C. 2015. Lion (*Panthera leo*) populations are declining rapidly across Africa, except in intensively managed areas. *Proc. Natl. Acad. Sci. USA* **112**: 14894–14899. [Medline] [CrossRef]
6. Benavides Valades, G., Ganswindt, A., Annandale, H., Schulman, M. L. and Bertschinger, H. J. 2012. Non-invasive assessment of the reproductive cycle in free-ranging female African elephants (*Loxodonta africana*) treated with a gonadotropin-releasing hormone (GnRH) vaccine for inducing anoestrus. *Reprod. Biol. Endocrinol.* **10**: 63. [Medline] [CrossRef]
7. Bertschinger, H. J., Guimarães, M. A. B. V., Trigg, T. E. and Human, A. 2008. The use of deslorelin implants for the long-term contraception of lionesses and tigers. *Wildl. Res.* **35**: 525–530. [CrossRef]
8. Bertschinger, H. J. and Lueders, I. 2018. Use of antigonadotropin-releasing hormone vaccines in African elephants (*Loxodonta africana*): A review. *Bothalia* **48**: a2320. [CrossRef]
9. Botha, A. E., Schluman, M. L., Bertschinger, A. J., Guthrie, A. J., Annandale, C. H. and Hughes, S. B. 2008. The use of a GnRH vaccine to suppress mare ovarian activity in a large group of mares under field conditions. *Wildl. Res.* **35**: 548–554. [CrossRef]
10. Dunshea, F. R., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K. A., Lopaticki, S., Nugent, E. A., Simons, J. A., Walker, J. and Hennessy, D. P. 2001. Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *J. Anim. Sci.* **79**: 2524–2535. [Medline] [CrossRef]
11. Imboden, I., Janett, F., Burger, D., Crowe, M. A., Hässig, M. and Thun, R. 2006. Influence of immunization against GnRH on reproductive cyclicity and estrous behavior in the mare. *Theriogenology* **66**: 1866–1875. [Medline] [CrossRef]
12. IUCN. 2020. The IUCN Red List of Threatened Species. https://www.iucnredlist.org/species/15951/115130419 [accessed on December 3, 2020].
13. Kawase, K., Ban, K. and Shiihara, S. 2019. A questionnaire survey of rearing management (contraception, feeding and access to an outdoor yard overnight) in big cats. *J. Jpn. Assoc. Zoo*. *Aqua* **60**: 97–107.
14. Kawase, K., Tomiyasu, J., Ban, K., Kimura, R., Ono, R., Saito, A., Matsui, M. and Shiihara, S. 2018. Minimally invasive monitoring of the estrous cycle based on changes in plasma progesterone concentration and vaginal cytology in a captive female Lion (*Panthera leo*). *Jpn. J. Zoo Wildl. Med.* **23**: 83–89. [CrossRef]
15. Khumsap, S., Thitaram, C. and Somgird, C. 2020. GnRH vaccine could suppress serum progesterone level in Thai pony mares; A preliminary study. *Vet. Integr. Sci.* **18**: 43–51.
16. Levy, J. K., Friary, J. A., Miller, L. A., Tucker, S. J. and Fagerstone, K. A. 2011. Long-term fertility control in female cats with GonaCon™, a GnRH immunocontraceptive. *Theriogenology* **76**: 1517–1525. [Medline] [CrossRef]
17. Lueders, I., Hildebrandt, T. B., Gray, C., Botha, S., Rich, P. and Niemuller, C. 2014. Supression of testicular function in a male Asian elephant (*Elephas maximus*) treated with gonadotropin-releasing hormone vaccines. *J. Zoo Wildl. Med.* **45**: 611–619. [Medline] [CrossRef]
18. Miyamoto, A., Okuda, K., Schweigert, F. J. and Schams, D. 1992. Effects of basic fibroblast growth factor, transforming growth factor-beta and nerve growth factor on the secretory function of the bovine corpus luteum in vitro. *J. Endocrinol.* **135**: 103–114. [Medline] [CrossRef]
19. Powers, J. G., Baker, D. L., Davis, T. L., Conner, M. M., Lothridge, A. H. and Nett, T. M. 2011. Effects of gonadotropin-releasing hormone immunization on reproductive function and behavior in captive female Rocky Mountain elk (*Cervus elaphus nelsoni*). *Biol. Reprod.* **85**: 1152–1160. [Medline] [CrossRef]
20. Putman, S. B., Brown, J. L., Franklin, A. D., Schneider, E. C., Boisseau, N. P., Asa, C. S. and Pukazhenthi, B. S. 2015. Characterization of ovarian steroid patterns in female African Lions (*Panthera leo*), and the effects of contraception on reproductive function. *PLoS One* **10**: e0140373. [Medline] [CrossRef]
21. Schulman, M. L., Botha, A. E., Muenscher, S. B., Annandale, C. H., Guthrie, A. J. and Bertschinger, H. J. 2013. Reversibility of the effects of GnRH-vaccination used to suppress reproductive function in mares. *Equine Vet. J.* **45**: 111–113. [Medline] [CrossRef]