Deslorelin as a contraceptive in female leopard geckos (*Eublepharis macularius*)

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Abstract

It is possible to chemically neuter several mammal species with Deslorelin implants. The big advantage of chemically neutering an animal over surgically neutering, is that chemically neutering is reversible. Deslorelin is a gonadotrophin releasing hormone (GnRH) agonist and therefore it stimulates the release of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). When administered over a long period of time, it will suppress the release of FSH and LH and stop ovulation in some female mammals. Contraception in reptiles is not widely used, but with an increase in reptiles kept as recreational pets, this might change. To research if reptiles can be chemically neutered, female leopard geckos were administered deslorelin implants (*suprelorin 4,7 mg from Virbac*) intracoelomic and their health was monitored after the procedure. The leopard geckos were then followed up on for 6 months to see if there was egg production. In this study 14 female leopard geckos (*Eublepharis macularius*) were used. In 9 female leopard geckos were given a deslorelin implant and 5 geckos were given a placebo implant. The time it took the geckos to recover from the procedure and the complications during the healing progress were also monitored. All 14 geckos recovered completely in 1-4 weeks (mean: 2.43, median 2.00, s.d. +- 0.756). The only complications that were seen, were mild (hematoma (6), some blood loss (4) and inflammation of the insertion wound (2)). After 6 months, all 14 geckos had laid eggs, both the geckos with a deslorelin implant and the geckos with the placebo implants. From these results, it can be concluded that it is safe to administer a deslorelin implant in a female leopard gecko, but that the implants do not prevent the geckos from laying eggs in the six months after administration.

Introduction

Contraception is widely used to control both domesticated and wild animal populations. There are different forms of contraception, the most widely known being surgically neutering an animal by removing the gonads, but this is non-reversible and invasive. Another form of contraception is chemically neutering an animal, which often is reversible. The animal is neutered with drugs instead of with surgery. Chemically neutering an animal can be done by repeated injections or implants. One of these implants, containing Deslorelin, has proven successful in dogs, house cats, big cats, ferrets and pigeons. [1-4]

Deslorelin is a nonapeptide analogue of gonadotrophin releasing hormone (GnRH). In mammal's, GnRH is produced pulsatile in the surge centre of the hypothalamus of the brain under neural influence. GnRH is transported to the anterior pituitary gland via the hypophyseal portal system. The anterior pituitary gland is then stimulated to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH stimulates the follicles in the ovary to grow and to secrete estrogens. LH then stimulates the follicles to develop further. LH also initiates ovulation and the forming of the corpus luteum. Then LH stimulates the production of estrogens, progesteron and inhibin by the corpus luteum. Estrogens inhibit both the release of GnRH by the hypothalamus and the release of FSH and LH by the anterior pituitary gland. Progesteron inhibits the release of GnHR and the release of LH. This negative feedback loop ensures that there is not a new ovulation during pregnancies.[5] This system is mostly the same in reptiles, with some differences in the composition of the GNrH molecules, the receptors in the brain and on the ovaries. Reptile behaviour can also vary from mammal behaviour under influence of gonadotrope hormones. The interactions between GNrH, FSH and LH seem to be the same for all vetebrates. [6, 7]

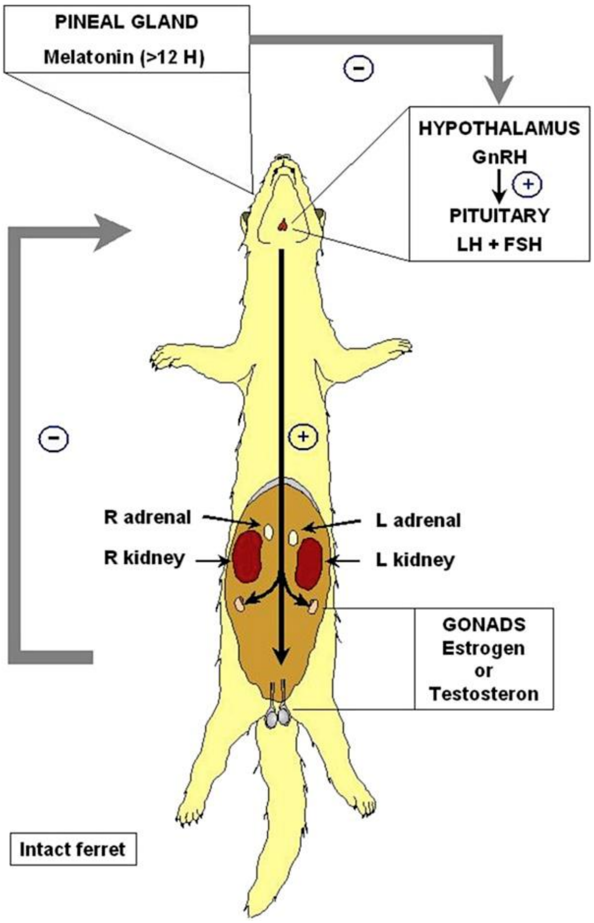

Image 1: the hypothalamic–pituitary–gonadal axis in an intact ferret. The hypothalamus produces GnRH, which stimulates the pituitary gland to produce LH and FSH. LH and FSH stimulate the gonads to produce estrogen or testosteron. Estrogen and testosteron suppress GnRH, LH and FSH production.[8]

Deslorelin is a GnRH agonist and when administrated in a single high dose it stimulates the release of FSH and LH and it can induce ovulation. When administered over a long period of time in a small dose it will, in several mammal species, suppress the release of FSH and LH. The suppression is likely a result from desensitisation of the receptors that are responsible for gonadotrophin release, a loss in binding capacity of the receptors on the pituitary and a depletion of pituitary gonadotrophins. Due to this suppressing mechanism, deslorelin can be used as a contraceptive in a lot of mammals.[9]

Leopard geckos (*Eublepharis macularius*) are carnivorous reptiles of the order of *Squamata*. They can be found in Asian dessert areas, from Pakistan to the north western part of India. Leopard geckos are very popular pets. They are easy to keep and they breed well in captivity, making them ideal animals for scientific research.[10] Leopard geckos are sexual dimorphic, making males and females of the species easily recognizable.

Image 2: sketch of the difference between male and female leopard geckos. On the left is the male, with hemipenile bulges and preanal pores. The female on the right lacks these characteristics.[11]

Females of the species have a single germinal epithelium on each ovary, with numerous oogonia and primordial follicles. In the stroma of the ovaria, follicles of different stages of development can be found.[12] When not receptive to mating, female leopard geckos have low levels of testosterone in their blood. These testosterone levels rise when the female is receptive, when she is in late vitellogenesis. After ovulation, the testosterone levels fall back to normal values. A female leopard gecko can be made receptive by administration of testosterone. In most reptiles, testosterone levels are, at all times, higher than oestrogen levels.[11] In leopard geckos, no research has been done on hormone levels, but in the Kemp's ridley sea turtle (*Lepidochelys kempi*), testosterone levels are 10 times higher than oestrogen levels, and testosterone levels are 20 times higher during the mating season. In this research, it was also found that Oestrogen levels rose, starting during previtellogenesis and peaking during late vitellogenesis. Progesterone levels rose during early stages of vitellogenesis but remain the same during late vitellogenesis. This could mean that testosterone and oestrogen have more impact on the behaviour of Kemp's ridley sea turtle than progesterone.[13] In the tegu lizard (*Salvator merianae*) hormone concentrations of oestrogen in females were high during the mating season (spring) and the progesterone levels were high in October, when the lizards are showing nesting behaviour.[14] This suggests that, just like in mammals, GnRH plays a role in ovarian activity in reptiles.

Contraceptives have been used in domesticated animals for a long time, but not often in reptiles. Pet reptiles are either kept solitary or they are kept for breeding purposes. Therefore, in a lot of cases, contraceptives were not considered necessary for pet reptiles. In recent years, reptiles have become more popular as pets for recreational owners instead of breeders. Certain problems in reptiles, like dystocia or aggressive behaviour, can be prevented or resolved when an animal is neutered. Dystocia is a problem often seen in chameleons and other lizards, which are popular pet reptiles.[15] A rise in need for contraceptives for reptiles can be expected. Examples of popular reptiles kept as pets that do not need to live solitary are leopard geckos, corn snakes, tortoises and iguanas. Surgically neutering reptiles can be done, but not many veterinarians offer these services. Surgically neutering is an invasive procedure, where the gonads are removed trough a wound in the skin and abdominal wall. An easier way of neutering reptiles would be with implants, especially considering it is easier to learn and train for most veterinarians than anaesthesia for and operating on reptiles. But not a lot is known about the effectiveness of chemically neutering reptiles.[16] Tests on the effectiveness of deslorelin implants in reptiles have been done in veiled chameleons (*Chamaeleo calyptratus*), green iguanas (*Iguana iguana)*, yellow-bellied sliders (*Trachemys scripta* sp) and red-eared sliders (*Trachemys scripta elegans*). In these tests, only in the green iguana, the ovarian activity was supressed. In the yellow-bellied sliders, a drop in testosterone was seen, 2 weeks after administration of the deslorelin implants, which corresponts with the initial surpesion often seen. However, After this initial drop, the testosterone levels returned to normal.[8, 17]

A study about the effectiveness of deslorelin implants in reptiles, could prove very useful for pet owners and veterinarians and provide more knowledge about contraception in reptiles. Leopard geckos are often kept as pets and are well suited for research purposes. Therefore, this paper aims to answer the question: 'Will female leopard geckos (*Eublepharis macularius*) no longer lay eggs when implanted with a deslorelin implant during 6 months after administration?' Because not only the contraception in reptiles, but also the practical application of the deslorelin implants in reptiles is important, another question arises: 'Will female leopard geckos survive the administration of deslorelin implants without severe complications?'

Material and methods

For this study 14 female leopard geckos (*Eublepharis macularius*) were used. Their ages ranged from 1 to 2 years old. All geckos were determined to be healthy, by checking their weight, growth rate, breathing frequency in rest, activity, appetite and by checking their faeces for parasites or undigested prey. All geckos were of age and weight to reproduce. The weight of the geckos was monitored once a week, from the moment they entered the test, until the day the test ended. The housing of the geckos consisted of glass terraria, divided by cardboard. The geckos were housed in groups of 3 or 4.

Image 3: a close up of one of the living spaces of the geckos.

The terraria were provided with a heath lamp and a UV-light. The geckos received light for 16 hours every day and the temperature was kept between 20 and 30 degrees Celcius. The geckos were randomly allocated into two groups, one group receiving deslorelin implants (*suprelorin 4,7 mg from Virbac*), registered for dogs, the other group receiving placebo implants. Both the researches and caregivers were unaware of which geckos received functional implants and which geckos received the placebo implants. The placebos were provided by the manufacturer of the deslorelin implants and were undistinguishable from the working implants. The manufacturer also provided a list with the registrations of all the implant and which of them were placebos. This list was not looked at before or during the experiment by anyone involved.

The Geckos were anesthetized (alfaxalone 15 mg/kg IM (Alfaxan® injection for dogs and cats, Vétoquinol)) and received analgesia (meloxicam 1 mg/kg IM (with either butorphanol 2 mg/kg IM (Torbugesic® Small Animals and Horse, Pfizer) or tramadol 10mg/kg IM (Tramal® injekcí roztok)) before being implanted with deslorelin 4,7 mg implants. The implants were administered intracoelomic with the applicator which is supposed to be used for dogs and which was provided together with the implants. The insertion was made slightly left or right of the midline, against the coelom lining behind the liver. Where deemed necessary, 1 or 2 interrupted, over and over sutures were used to close the puncture wound. Non-resorbing, monofilament material 4-0 was used. Since the geckos will shed the skin with the sutures after some time, they did not have to be removed manually.

Image 4: the location of the insertion wound. This picture was taken after the wound had closed and some scar tissue has formed after 3 weeks.

During the procedure, the breathing frequency and the body temperature were monitored. After the procedure, the female geckos were housed in terraria in groups of 2 – 4 geckos, depending on their size and temperament. Together with the female geckos, two male geckos were also kept in the terraria, but they had no direct access to the female geckos. The presence of a male gecko stimulates the female geckos to lay eggs. Because the male had no access to the females, the eggs would not be fertilised.

After the procedure, the female geckos' wounds were checked twice a week for bleeding, swelling, shedding of the sutures, closing of the wound and formation of scar tissue. The checking of the wound was stopped after it had healed. The overall health of the geckos was monitored through their weight, appetite and their state of hydration. With transillumination the ovaria were checked for eggs. Transillumination was done by shining a strong light dorsoventrally trough the geckos abdomen in a darkened room. This method makes certain organs, like the liver, visible through the skin of the gecko as dark objects. A female usually develops two eggs in the ovaria, which are visible before she lays the eggs.

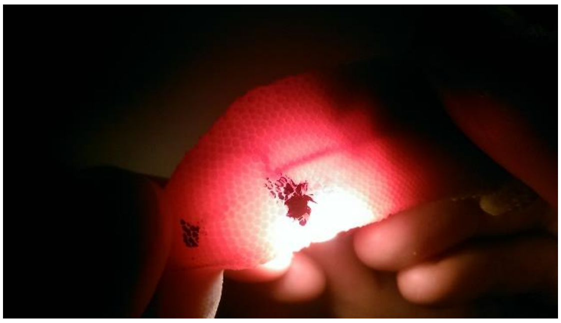

Image 5: transillumination of the abdomen of a female leopard gecko, after the deslorelin implant was administrated. The dark spot in the centre is dried blood on the skin, the red line trough the illuminated part, is the dorsal aorta. No ovaria or developing eggs are visible on this image.

The nesting places in the terraria were checked for eggs. When a female who previously had opaque objects in the coelom, did not have them anymore after eggs had been found, the assumption was made that that female had lain the eggs. Female leopard geckos lay cludges of 2 eggs at a time and they lay multiple cludges in each nesting season.[18] The geckos were monitored for 6 months. After 6 months, it was possible to determine which gecko had a working implant and which gecko had a placebo implant through the registration of the batch numbers and the list provided by the manufacturer.

Results

The female leopard geckos underwent the surgery without problems. All 14 geckos shed their suture without major complications. The time it took the geckos to shed the sutures ranged from 1 to 4 weeks (mean: 2.43, median 2.00, s.d. +- 0.756). (See table 1). Some of the minor complication that were seen were hematoma (6), some blood loss (4) and inflammation (2). All these complications were minor and were either left untreated or treated with a topical iodine solution where inflammation was seen or when there was a risk of inflammation.

Image 6: hematoma seen shortly after the procedure in one of the geckos.

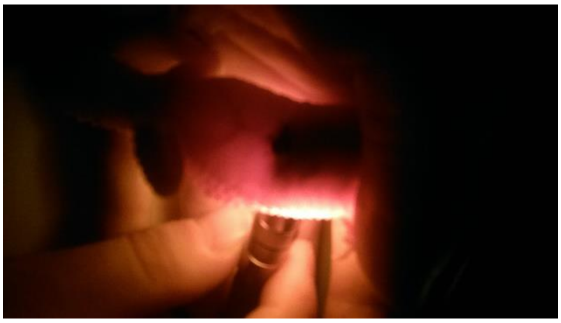

Image 7: the same gecko with a hematoma seen trough transillumination.

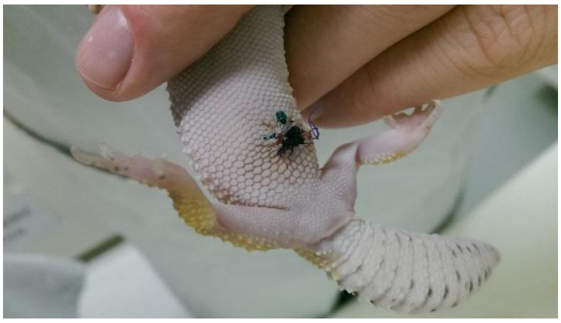

Image 8: a gecko with some inflammation around the sutures, as seen by the dark red outlines between the white scales around the wound. The blue/green product on the suture is an iodine solution.

All 14 geckos produced eggs between 1,5 months and 5 months after administration of the deslorelin implant or the placebo implant. This was confirmed with transillumination and the finding of eggs in the terraria. 13 geckos produced multiple cludges of eggs. No difference was seen in deslorelin implant group and the placebo implant group. (See table 1) A chi-square test could not be done, because there were no geckos that did not lay eggs. This left one of the groups of values to be compared by a chi-square test empty, meaning there was nothing to compare with the test.

Has laid eggs		Deslorelin implant	Placebo implant	total
yes	14	9	5	14
no	0	0	0	0
	total	9	5	14

Table 1: distribution of which geckos have laid eggs in which group. Every gecko has laid eggs. A chi-square test could not be done, because there are no values in the group of geckos that did not lay eggs. Therefore, a comparison of values could not be done.

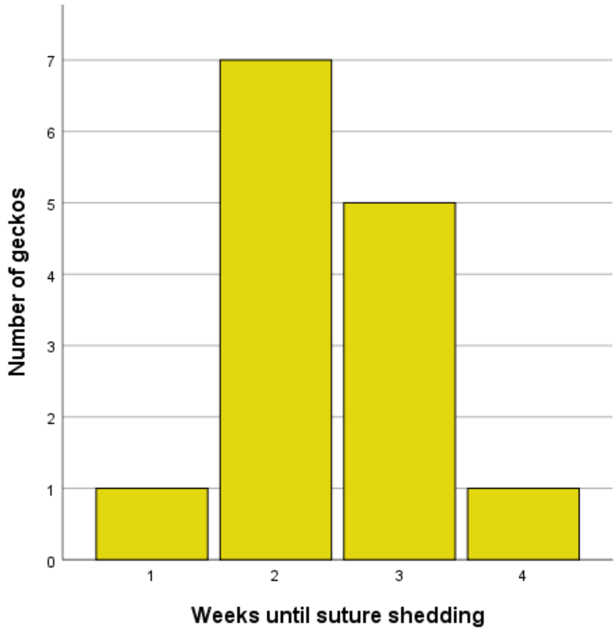

Figure 1: The number of geckos that shed their sutures in 1, 2, 3 or 4 weeks after implant administration. A Shapiro-Wilk test shows the normality of the results with a significance of 0.032. (mean: 2.43, median 2.00, s.d. +- 0.756).

Discussion

To answer the question 'Will female leopard geckos survive the administration of deslorelin implants without severe complications?' the geckos were monitored for complications and the time it took for them to shed the sutures that were administered during the procedure. Since the deslorelin implants were designed for use in dogs, they were rather large for a leopard gecko. All geckos survived both the administration of the implant and the healing period after. The sutures were mostly shed in 2 to 3 weeks without major complications. The complications that were seen included: inflammation, blood loss and haemorrhages. With a topical iodine solution for the inflammation, these complications healed and the geckos recovered without further problems. With these results, it is safe to say the procedure to administer deslorelin implants in the coelom is safe in leopard geckos.

Female leopard geckos still lay eggs, even when they have a deslorelin implant administered to them. When administering a deslorelin implant as a contraceptive, a high rate of effectiveness is important. Even a small chance of it not working, could be a reason to not use it. It did not work in any gecko in this test, therefore it can be concluded that deslorelin does not work effectively as a contraceptive in female leopard geckos in the six months after administration. To find the reason why deslorelin does not seem to work in female leopard geckos, more research must be done, but a couple of explanations can be thought of.

First, the hormones that influence the ovulation of geckos can differ from those in mammals. As stated before, testosterone plays a much bigger role in ovulation in leopard geckos than it does in mammals. It could be that depressing testosterone in leopard geckos might stop ovulation more effectively than depressing gonadotrophin releasing hormone (GnRH).

Secondly, the implants might not behave the same in leopard geckos as in mammals. The deslorelin implants used in the test were designed for dogs and were thus meant for much bigger animals than leopard geckos. How this affects the workings of the implant is unknown, but it could lead to different dissolving rates. The implants were also designed for subcutaneous administration. The implants were now administered intracoelomic, but this might affect the way the deslorelin is absorbed in the body. The implant could dissolve much faster or not at all. The implants might also be encased in body tissue, as protection of the body against foreign objects.

To get more information on the dissolving and absorption of the deslorelin implants, the implants should be recovered after a certain time, like 6 months or a year. Blood tests for hormones like GnRH, FSH and LH could regularly be done before and after administering the deslorelin implant to monitor the effect of the implants. This is however very difficult to do in leopard geckos, since they are small (20-40g) and therefore are hard to extract blood from.[19] It would be better to choose a larger reptile (for example the green iguana (*Iguana iguana*) or the ball python (*Python regus*)) for regular taking of blood to measure the hormone levels.

Other options to get more information about the effects of deslorelin implants in leopard geckos could be to prolong the test to more than 6 months. A different dosage could also be looked at.

In conclusion: in this study the deslorelin implants did not work effectively as a contraceptive in female leopard geckos in the six months after administration, but the procedure to administer the implants was safe. To find out why deslorelin does not work or to find an effective contraceptive for reptiles, more research is needed.

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