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Research article

Effect of deslorelin implants on the testicular function in male ring-tailed lemurs (*Lemur catta*)

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Abstract

Ring-tailed lemurs (*Lemur catta*) are popular exhibit animals in zoos. During the breeding season, males may fight and this can result in serious wounds and/or escapes. The hormonal contraceptive deslorelin, a GnRH-agonist, has been used in different species to suppress reproduction. This contraceptive strategy can reduce the production of sexual hormones and, therefore, could be used to control aggressive behaviour. Here, we analysed the effect of a 4.7 mg deslorelin implant on testicular function in five male ring-tailed lemurs. The aim of the study was to assess if this contraceptive strategy could be used to reduce testosterone levels and thus aggressive interactions between individuals. Neither testosterone concentrations detected in faeces nor spermatogenesis evaluated by testes histology was suppressed by the deslorelin treatment. These results suggest that a GnRH implant containing 4.7 mg deslorelin has no contraceptive effect in ring-tailed lemurs. The effect of the use of different dosages of deslorelin implants, as well as that of other hormonal contraceptives, should be evaluated in this species.

Introduction

Ring-tailed lemurs (*Lemur catta*) live in multimale-multifemale groups usually numbering six to 24 individuals. Females are dominant over males, but there is also a dominance hierarchy among males. In the wild, males become sexually mature at about three years of age and some emigrate from the group at this age (Gould and Ziegler 2007). In captivity, males may fight during the breeding season, and this may result in serious wounds and/or displaced males escaping out of the enclosure. This aggressive behaviour in male ring-tailed lemurs has been positively correlated with testosterone levels during the mating season (Cavigelli and Pereira 2000). Likewise, to control aggressive interactions in domestic animals (Vinke et al. 2008; Lucas 2014), castration of mature males has been used as a management tool in ring-tailed lemurs (Fernandez-Bellon et al. 2013). Nevertheless, the sudden drop of testosterone concentrations in some of the adult males, due to surgical castration, may have a negative effect on group dynamics. One alternative approach for contraception is to use a gonadotropin-releasing hormone agonist (GnRH-agonist) to suppress the hypothalamic-pituitary-gonadal (HPG) axis activity and thus reduce testosterone levels and spermatogenesis gradually. GnRH-agonists can also be used as a management strategy before performing surgical castration, in order to avoid the sudden drop of testosterone concentrations.

The gonadotropin-releasing hormone (GnRH) is a hypothalamic hormone that plays a key role in the physiology of reproduction with the same amino acid sequence in all mammals (Lucas 2014). GnRH-agonists induce similar actions as the endogenous GnRH (Penfold et al. 2002). The pulsatile release of the native GnRH is responsible for the stimulation of LH and FSH secretion; whereas the non-pulsatile, continuous release of an exogenous GnRH-agonist is known to prevent, rather than stimulate, gonadotropin release, believed to be due to the down-regulation of GnRH-receptors. Paradoxically, the initial binding of the GnRH-agonist provokes an additional GnRH release (positive feedback) but is soon followed by a

gradual inhibition of the native pulsatile GnRH secretion (negative feedback). Consequently, a decline in gonadotropin secretion is observed, which, ultimately, reduces testosterone concentration (Aspden et al. 1997). Therefore, GnRH-agonists can potentially be used to solve animal aggressive behaviours triggered by high circulating concentrations of testosterone.

The GnRH-agonist deslorelin, a synthetic biodegradable GnRH analogue, has been used for different purposes in a variety of domestic and non-domestic species (Penfold et al. 2002; Lee et al. 2014; Lucas 2014; Raines and Fried 2016). Many studies have demonstrated the implant's safety and its well-tolerated antifertility effects since Trigg and colleagues (2001) performed the first successful experiment on dogs. The use of deslorelin is recommended by the Association of Zoos & Aquariums Reproductive Management Center (AZA RMC) for prosimians (https://www.aza.org/reproductive-management-center); nonetheless, no specific studies have been performed regarding its use in ring-tailed lemurs.

In the present study, the effect of a deslorelin implant on the testicular function in five male ring-tailed lemurs was analysed with the aim to assess the reduction in testosterone levels, and thus control inter-male aggressive behaviours. For this purpose, faecal metabolites of testosterone concentrations were monitored during the study. At the end of the evaluation, the animals were castrated and a histological examination of the testicles was conducted to assess spermatogenesis.

Methods

Animals and study design

Nine male ring-tailed lemurs (*Lemur catta*), from the Bioparc Valencia in Spain, were randomly assigned to treatment (n=5) and control group (n=4). The treatment group was composed of five males, individually housed in indoor boxes of 6 m^2 each, four of which were sexually mature (Table 1). The control group comprised four sexually mature males housed together in an outdoor area of 30 m^2.

All GnRH treated males, after a period of 161 days (31st of May until 5th of October 2013), were orchidectomised and their testicles were processed for histological evaluation. Faecal samples were collected from treated and control groups, starting one week before implant administration to one week before castration, in a 21-day periodicity schedule. All animals were kept in closed facilities according to established husbandry protocols for this species (Puchmann 2004) and they were fed on a regular lemur diet twice a day. Water was provided ad líbitum.

Table 1. Identification (ID) and age of treated lemurs during the study

ID	Date of Birth	Age at start of study (years)
#1	02/03/2006	7
#2	15/05/2007	6
#3	02/04/2009	4
#4	05/04/2009	4
#5	14/05/2011	2

Deslorelin implantation and castration

Animals from the treatment group were anesthetised following established veterinary protocols. A GnRH implant, containing 4.7 mg deslorelin was inserted subcutaneously in the neck of each anesthetised male. After 23 weeks, following surgical castration of all treated males, the testicles were processed for histological examination. Implants were left in place and not removed after castration.

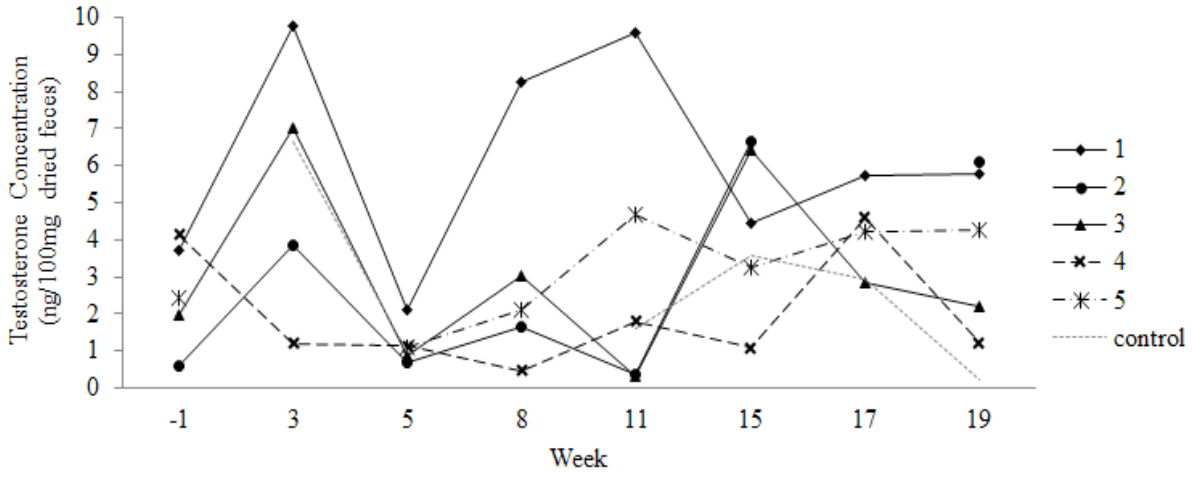

Figure 1. Faecal testosterone metabolite concentrations for treatment (#1 to #5) and control groups. Pre-treatment hormone concentrations were obtained during week −1. Deslorelin implantation was performed at week 0 (between week −1 and week 3). Castration was performed between week 17 and week 19. Lack of sample collection for ID #2, #5 and control are shown with interrupted lines.

Histological examination of the testicles

Immediately after castration, both testicles and epididymal sections were fixed in buffered formalin for at least 24 h. Further specimen processing included samples being dehydrated, embedded in paraffin, then sectioned into 5 µm slices, stained with haematoxylin-eosin, and analysed under light microscopy.

Faeces collection and testosterone metabolite analysis

A minimum of 300 mg of fresh faecal samples were collected with rubber gloves and placed in a labeled polypropylene vial. Faecal samples were collected between 0730 and 0830 directly following defaecation and were immediately frozen at –20°C.

Faeces were prepared and assayed following a methanol extraction protocol previously described (Sabés-Alsina et al. 2015; Tallo-Parra et al. 2015). Faecal testosterone metabolite measurements were performed using a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA) (DetectX® Testosterone Immunoassay; Arbor Assays, Eisenhower Place, USA) with a specifically generated antibody to measure testosterone and its metabolites in urine and faecal samples.

Statistical analysis

A repeated measures ANOVA was performed in R software (R Development Core Team, University of Auckland, New Zealand; package lme4) to analyse testosterone differences across weeks and among individuals. When significant, multiple comparisons of group means were assessed using the Tukey's test. A P-value below 0.05 was established as a criterion for significance.

Hormone concentrations

Five weeks after implant administration, faecal testosterone concentrations decreased significantly compared to the previous faecal analysis on week 3 after implantation ($P<0.05$; Figure 1). When analysing variation among individuals, only animal ID #1 showed significant differences compared to ID #2, ID #3, ID #4 and control group ($P<0.05$).

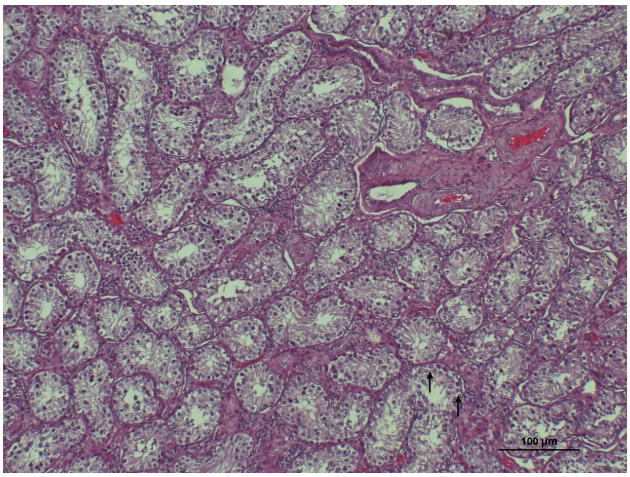

Figure 2A. Haematoxylin-eosin staining of the male ID #5 testicle showing the compact seminiferous tubules without lumen and some spermatogonia (arrows) at the base of the tubules. Scale bar=100 µm.

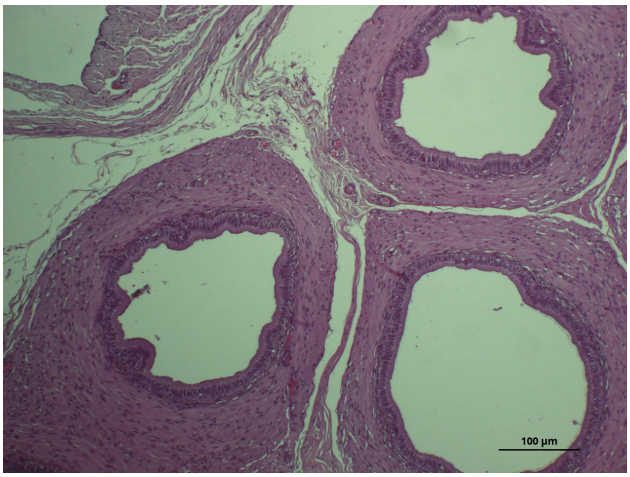

Figure 2B. Haematoxylin-eosin staining of the male ID #5 epididymis. No presence of sperm cells was observed in this anatomical structure. Scale bar=100 µm.

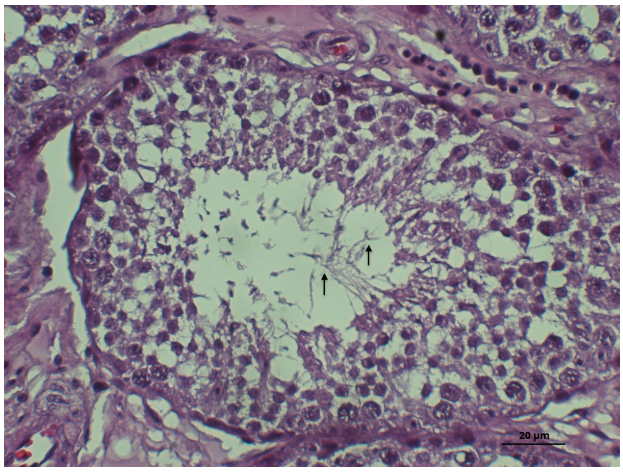

Figure 3A. Haematoxylin-eosin staining of the male ID #3 testicle. Image shows the presence of spermatids (arrows) in the seminiferous tubes. Scale bar=20 µm.

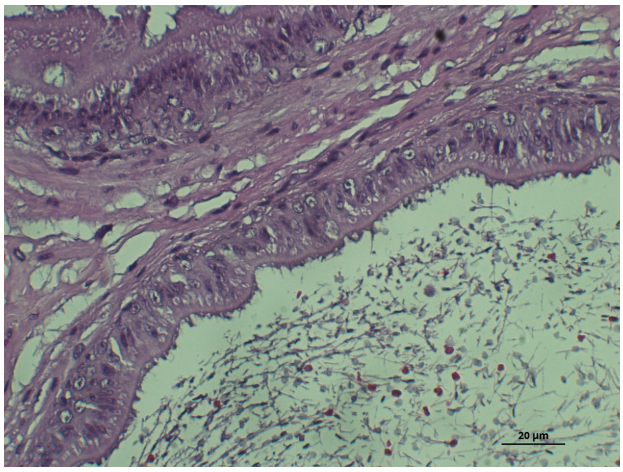

Figure 3B. Haematoxylin-eosin staining of the male ID #3 epididymis with presence of sperm cells in the lumen. Scale bar=20 µm.

Results of the histological examination

In the histological evaluation, the testicles of the youngest male, ID #5, did not show active spermatogenesis. Its seminiferous tubules were compact, without lumen and spermatogonia were only detected at the base of the tubes (Figure 2A). No sperm cells were detected in the epididymis (Figure 2B). The individual ID #4 showed moderate spermatogenesis, with spermatocytes in the lumen but without the presence of mature spermatozoa in tubes, or epididymis. The remaining three males, ID #1, #2 and #3, showed active spermatogenesis with abundant spermatozoa in tubes and epididymis (Figure 3A and 3B).

Discussion

To the authors' knowledge, this is the first study to document the use of a GnRH-agonist and its reproductive effects in ring-tailed lemurs.

Importantly, a continued production of testosterone was observed in the GnRH-agonist implanted males through the analysis of faecal testosterone metabolites, which in turn could have maintained the spermatogenic activity, as shown by the histological examination in four of the five treated animals. Although intermittent peaks of testosterone and its metabolites were detected during almost the entire treatment, differences in faecal hormone concentrations between the third and the fifth week after implantation were observed. Concretely, testosterone concentrations decreased in the fifth week compared to the previous faecal analysis in individuals ID #1, #2, #3 and controls. As previously described in other mammal species (Penfold et al. 2002; Herbert et al. 2004), before exerting the chronic negative feedback, GnRH-agonists hyperstimulate the GnRH receptors in the pituitary gland causing a testosterone peak (Ottinger et al. 2002). The hormone differences seen in the present study could be related to this stimulatory phase caused by the GnRH-agonist; however, statistical differences were also observed in the control animals. Thereby, whether the sudden testosterone drop detected was due to the GnRH-agonist or due to other individual causes remains to be explored. Despite this initial result, there was no evidence of suppression of gonadal activity during treatment. In addition, as confirmed with histology, spermatogenesis remained functional in most of the lemurs treated.

Additionally, variation in individual responses was observed with the oldest male (ID #1) showing significant differences in faecal testosterone concentrations compared to the other treated males (ID #2, #3 and #4). Higher circulating testosterone levels have been detected in older lemur males during the mating season (Gould and Ziegler 2007), explaining the potential differences observed in the oldest animal studied here.

Overall, our study has shown that a GnRH implant containing a 4.7 mg deslorelin treatment, following the manufacturer's recommended dosage, has no contraceptive effects in ring-tailed lemurs. Some experiments conducted in other species with the same drug and dose have shown effective responses (sea otters, Larson et al. 2013; pigeons, Lee et al. 2014; companion animals, Lucas 2014), although the use of higher dosages in wild animals is strongly recommended by the AZA RMC. Therefore, the use of higher dosages of deslorelin implants as well as the effect of other hormonal contraceptives should be evaluated in ring-tailed lemurs. Combining different endocrinological assessments, such as serum LH, FSH and testosterone quantification with histological exams, could help to understand the mechanisms of action and effects of contraceptive strategies in ring-tailed lemurs.

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