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GnRH-agonist implantation of prepubertal male cats affects their reproductive performance and testicular LH receptor and FSH receptor expression

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ABSTRACT

This study was conducted to investigate the effect of GnRH-agonist implantation in prepubertal tomcats on sexual behavior, reproductive performance, and expression of testicular LH receptor (LHR) and FSH receptor (FSHR) and also to compare the testicular characteristics, LHR and FSHR expression between prepubertal and adult tomcats. In experiment 1, 3-month-old tomcats (n = 6/group) were either treated with or left without 4.7 mg deslorelin implants. Semen collection and evaluation were performed just before castration at 48 weeks after treatment; removed testes were analyzed for mRNA and protein expression of LHR and FSHR. We were able to collect semen from six non-treated cats, whereas in treated cats, semen was uncollectable. The results revealed that sexual behavior was absent in the implanted cats throughout the study period. Testicular volume was found to decrease from 30 weeks after treatment onward in the implanted cats compared to the controls ($P < 0.05$). Semen production was found only in non-implanted cats. Testicular tissue score, seminiferous tubule diameter, and LHR protein expression were found lower in the implanted cats ($P < 0.05$), but no differences were observed in mRNA expression of *LHR* and protein expression of FSHR between groups. The mRNA expression of *FSHR* was higher in the implanted ($P < 0.05$) compared to control cats. In experiment 2, testes from prepubertal (n = 6) and adult (n = 6) male cats were collected after castration and analyzed for mRNA and protein expression of LHR and FSHR. No differences were observed in the protein expression of LHR and FSHR between the two groups, whereas mRNA expression of *FSHR* was higher in prepubertal cats ($P < 0.05$). Testicular and epididymal weight, diameter of seminiferous tubules, and the testicular grade were higher in the adult compared to prepubertal cats ($P < 0.05$). In conclusion, deslorelin implants suppressed protein expression of LHR and enhanced mRNA expression of *FSHR* along with suppression of reproductive function without any adverse effects for at least 48 weeks in male cats.

1. Introduction

Overpopulation of cats is a serious global problem, and in big towns/cities, roaming of tomcats is reported to be out of owners' control. The result is unwanted pregnancies with undesirable consequences in this species. Free-roaming cats

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without any care are also subjected to higher risks such as suffering from diseases (including zoonotic diseases), malnutrition, and accidents. The number of cats euthanized in shelters is also on the rise every year mainly due to overpopulation [1], which needs to be controlled to address the welfare problems associated with it.

Contraception is one of the most successful methods for population control in many animal species. Traditional way of contraception by castration is presently in practice in cats as well. However, castration is an invasive surgical procedure and can only be performed on anesthetized animals, whereas anesthesia poses serious problems in juvenile and senile cats and in cats with health problems. Cats reach puberty by the age of 4 months [2] with a possibility of mating soon after. However, surgical neutering in early age may pose risks such as higher sensitivity to many drugs including the anesthetics [3]. Therefore, nonsurgical neutering could be a welfare-friendly and viable alternative to surgical methods of neutering [4].

Reproduction in mammals is controlled by the hypothalamic–pituitary–gonadal (HPG) axis, and it has been shown that long-term continuous administration of GnRH desensitizes/downregulates the pituitary gland, profoundly suppresses the gonadotropins release, and impairs the reproductive function [5]. Accordingly, a contraceptive method has been developed; it is used by GnRH-agonist implantation (Suprelorin; Virbac, Carros, France) and has been proven effective in pubertal tomcats [5,6] and female domestic cats [6–8]. This method results in long-term reversible contraception without any negative effects to the animals. The contraceptive effects of GnRH-agonist have also been reported in other species such as dogs, wild felids, gilts, flying fox, and giraffes [9–15]. Moreover, Trigg et al. [16] have reported that when 4-month-old female pups were implanted with 9.4 mg of deslorelin, contraceptive effect was prolonged and lasted for at least 36 weeks, whereas the contraceptive effect in pubertal dogs was varied from 24 to 48 weeks. It is a possibility that this longevity effect might have been achieved by a delay in the age of puberty in these animals. Moreover, there are reports to suggest that early-age neutering could reduce undesirable behavior of cats especially in adopted cats and could help reduce the unwanted litters in many pet shelters. Although GnRH implantation has been used in cats to suppress the reproductive function, the studies in prepubertal cats are rare and with variable results [17,18].

The effects of GnRH implantation on the gonadotropins' release along with the suppression of reproductive function are well documented [5,19]. However, it is not known whether such effects are achieved through an alteration in the gonadal expression of receptors for LH and FSH and/or testosterone production. The present study was, therefore, designed to investigate the effects of long-term GnRH implantation (4.7 mg GnRH-agonist [deslorelin]) on the reproductive performance, testicular morphology, and expression of LH receptor (LHR) and FSH receptor (FSHR) in prepubertal male cats. Testicular morphology and expression of LHR and FSHR were also compared between prepubertal and adult male cats.

2. Materials and methods

2.1. Experiment design and animals

2.1.1. Experiment 1

Tomcats aged 3 months old that were proven to be clinically healthy and had attended a complete vaccination program were either implanted with 4.7 mg of deslorelin GnRH-agonist (Suprelorin 4.7 mg; Virbac Animal Health, France) in the interscapular area (deslorelin implanted; $n = 6$) or left without any implantation and served as controls (non-implanted; $n = 6$). The cats were housed together in an open-air room with natural daylight in the Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Thailand. During the study period, animals were fed with a commercial diet twice daily with water always available *ad libitum*. The study had ethical approval and was performed under the license of Chulalongkorn University Laboratory Animal Center (number 13310056).

Implanted animals were monitored for any potential adverse effects such as tissue reaction at the implantation site and/or infection, rashes, edema, erythema of implantation area, and so forth for a period of 1 week. Body temperature was measured daily for 1 week after the hormonal implantation to monitor any infection and if found, blood was collected for profile monitoring.

Body weight of all the cats in both the groups was recorded fortnightly until the end of the experiment (48 weeks) when both the testes were collected after surgical castration. Throughout the experimental period, functional evaluations of the reproductive organs such as penile spines, testicular volume and consistency, and male sexual behavioral characteristics were monitored at 2-week intervals in all the cats. Presence of penile spine was taken as a criterion of puberty [5]. Length, width, and depth of the scrotum/testis were measured using vernier calipers, and testicular volume was calculated with a modified spherical equation; volume (cm^3) = $4/3 \times \pi \times (1/2 \text{ length} \times 1/2 \text{ width} \times 1/2 \text{ depth})$ [5]. Testicular consistency was recorded by palpation by one observer and was noted as soft, firm, or hard. Male sexual behaviors such as marking, mounting (with or without intromission), and fighting [20] were observed for at least 30 minutes at 2-week intervals in all the cats. Feces were collected at 2-week intervals to measure testosterone concentrations. An attempt was made to collect semen from all the cats before surgical castration by using the electroejaculator, which was performed 48 weeks after implantation. Soon after collection, semen was evaluated for its volume, color, motility, concentration, viability, and sperm morphology. If semen ejaculation could not be accomplished, epididymal sperms were collected immediately after castration and evaluated.

2.1.2. Experiment 2

Testes were collected from 4 to 6-month-old (prepubertal, $n = 6$) or 1 to 3-year-old (adult, $n = 6$) normal healthy male cats after surgical castration at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand.