Chapter 10

Current and Future Options for Non-Surgical Neutering of Ferrets (*Mustela putorius furo*)

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Submitted

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Summary

Female ferrets (jills) are commonly ovariectomized to prevent estrus-induced bone marrow suppression, and male ferrets (hobs) are castrated to reduce intraspecies aggression and skin odor. There is increasing evidence, however, that castration may precipitate the development of hyperadrenocorticism in ferrets. The detection of luteinizing hormone receptors in the adrenal cortex of ferrets with hyperadrenocorticism has strengthened this notion. Since surgical castration is a major risk factor for the development of hyperadrenocorticism in ferrets, this paper concentrates on the current and possible future options for neutering ferrets. Some of the alternatives, such as progestagen administration, seem very practical in jills but the effect in hobs is uncertain. Possible future alternatives may be the use of slow-release gonadotropin-releasing hormone (GnRH) implants, GnRH antagonists, or immunization against GnRH.

Introduction

Surgical castration of ferrets (*Mustela putorius furo)* is common practice in the USA and various European countries. Female ferrets (jills) are induced ovulators and therefore remain in estrus until they are mated, or for as long as daylight lasts longer than 12 hours. In the early 1980s several publications appeared concerning estrogen-induced bone marrow suppression in jills with prolonged oestrus.[8,36,43,66] Since then, preventive ovari(ohyster)ectomy of jills has been advised. In male pet ferrets (hobs) there is no medical need for castration. The main reason to castrate hobs is to reduce aggression so that they can be kept in groups, and to decrease the intensity of the musky odor produced by the sebaceous glands.[51] In the USA it is common practice to castrate ferrets at 6 weeks of age, before their delivery to pet shops.[58]

Hyperadrenocorticism is a common disease among pet ferrets and is characterized by signs of excessive production of sex steroids (androstenedione, 17α-hydroxyprogesterone, dehydroepiandrosterone sulfate and/or oestradiol), i.e., symmetrical alopecia, vulvar swelling in neutered jills, and recurrence of sexual behavior in neutered ferrets.[40,57,58,63,71] In recent years, evidence has accumulated that hyperadrenocorticism in ferrets is mediated by an increased secretion of gonadotropic hormones after castration.[40,58,63] First, the initial signs of hyperadrenocorticism occur only during the breeding season,[56] when plasma concentrations of gonadotropic hormones are high.[33] Second, in the USA and in The Netherlands, where the neutering of ferrets is common practice, hyperadrenocorticism is a common condition.[56,63] In contrast, hyperadrenocorticism is seldom diagnosed in the United Kingdom, where ferrets often remain entire.[41] Third, a significant correlation has been found between the age at neutering and age at onset of hyperadrenocorticism.[63] Fourth, the gonadotropin-releasing hormone (GnRH)-analogue leuprolide acetate has recently been reported to have beneficial effects in the treatment of this disease.[70] Finally, luteinizing hormone (LH) receptors have been detected in the adrenal cortex of ferrets.[64]

Because several lines of evidence point to surgical castration as a major risk factor in the development of hyperadrenocorticism in ferrets, this paper concentrates on current and future options for the neutering of ferrets.

Reproductive physiology

Gonadal activity is seasonal in both male and female ferrets, and more than 12 hours of light per day promotes reproductive activity.[10,25] The pineal hormone melatonin plays a central role in the regulation of these changes,[27] and plasma and pineal gland concentrations of melatonin are significantly higher during the dark phase of the day (scotophase) than in the light phase (photophase).[7]

Plasma concentrations of follicle-stimulating hormone (FSH) increased within a few days after male hamsters were transferred from a short to a long photoperiod. Plasma LH concentrations, however, increased only after exposure to a female hamster.[2] The different regulation of LH and FSH secretion can be explained by the fact that there are at least two types of GnRH receptors [30] and several GnRH isotypes, some of which may have specific FSH-releasing activity.[72] Anand *et al.* also found that both melatonin and a GnRH-antagonist (antide) could prevent the release of LH in male hamsters exposed to a female.[2]

These findings indicate that melatonin suppresses the release of GnRH in seasonal breeders.

During the breeding season, GnRH stimulates the production of the gonadotropic hormones LH and FSH, which stimulate the gonads to produce either oestradiol or testosterone. The latter two hormones exert a negative feedback on the hypothalamus and pituitary gland, thereby preventing excessive secretion of GnRH, LH, and FSH.

Definitions

In its classical definition, castration denotes the removal of gonads. Thus the term covers both the removal of the ovaries (ovariectomy or spaying) in females and the removal of the testes (orchiectomy) in males.[3] In recent years, this definition has been extended by the introduction of new methods to create non-functional gonads, e.g., chemical castration and immunological castration.[5] The term castration is controversial since it is sometimes used to indicate the removal of the gonads in male individuals only.[16] A term that encompasses all means of eliminating gonadal function in both males and females is neutering,[4] and this term is used in this article.

There are methods of contraception that do not affect gonadal function. For example, fertilization of oocytes can be prevented by vaccination against zona pellucida proteins [6,26,44] or vaccination against sperm proteins.[39,53] Another option to prevent oocyte fertilization is surgical sterilization, by means of tubal ligation. Immunization of female rats and monkeys with riboflavin carrier protein caused termination of pregnancy around the pre-implantation stage, while immunization of male rats and monkeys resulted in a reduced fertilizing potential of their spermatozoa.[1] Since these methods do not eliminate gonadal function and therefore do not prevent the resulting detrimental effects in ferrets, they are not discussed here.

Progestagens

Principle: Although not fully understood, the probable mode of action of progestagens is suppression of the secretion of gonadotropic hormones, thereby preventing ovarian cyclicity.[15]

Method: Several progestagens are used in veterinary medicine: medroxyprogesterone acetate, megestrol acetate, and proligestone. Megestrol acetate (Ovaban; Schering Plough) can be given orally, medroxyprogesterone acetate (Depo-provera; Pharmacia) can be given orally or by injection and proligestone (14α, 17α propylidene-dioxy progesterone) (Delvosteron; Intervet) is given by depot injection. The latter is recommended in the United Kingdom for the prevention of estrus in ferrets at a dose of 0.5 ml (100 mg/ml SC) just prior to the breeding season.[35,52] Proligestone can also be used in jills in estrus.

Effect: Return of estrus was reported in approximately 8% of ferrets 2 – 5 months after the initial dose of proligestone. In these cases, a second dose suppressed estrus for the rest of the breeding season.[52] Megestrol acetate has been used in ferrets to prevent estrus but is not recommended because of the assumed risk of pyometra.[19,60] However, this restriction is probably not justified because pyometra has not been described in ferrets after the use of megestrol acetate.[17,29]

Remarks: Reported side-effects associated with the use of progestagens in either dogs or cats are the development of cystic endometrial hyperplasia (CEH), prolonged pregnancy, hypersecretion of growth hormone (GH), diabetes mellitus, and increased risk of neoplastic transformation of mammary tissue.[62] Of these side-effects, only prolonged pregnancy (gestation 51 days, normal 38 – 44 days) has been reported after the use of proligestone. Proligestone had been given to these two ferrets when they were in estrus and had been mated.[35]

There is no information on the use of progestagens in hobs. In other species, including humans, progestagens have been used to suppress libido and fertility in males.[23,47,67] Progestagens are rarely used for contraception of human males since they cause loss of libido and incomplete suppression of spermatogenesis.[47] For this reason the combination of progestagens and androgens is often used: it provides a better contraceptive effect than progestagens alone and the libido is maintained.[22,47] This combination would not be an option in ferrets, because libido is an undesirable characteristic in hobs. Delmadinone acetate (Tardak; Pfizer) is used to suppress libido in dogs.[67] A recent study of Beagles, however, has revealed that this progestagen does not suppress plasma testosterone concentrations.[38] Thus delmadinone acetate, when used in ferrets, may not suppress the musky odor produced by the sebaceous glands. In humans, cyproterone acetate (Androcur; Schering) and medroxyprogesterone acetate have been used to suppress libido in sex offenders.[23] Both drugs suppress plasma testosterone concentrations.

Studies with progestagens are needed to determine whether these drugs can be used to control libido and odor in hobs. In addition, the effect of progestagen administration on GH release should be studied in jills, because progestin-induced expression of the mammary GH gene has now been demonstrated in dogs, cats, and humans.[49,50,65]

"Sham" mating

Principle: Ferrets, rabbits, and cats are all induced ovulators. When ovulation is achieved without fertilization, pseudopregnancy will occur.[19]

Method: In rabbit does, the proximity of an intact male, mechanical stimulation of the vagina, or mounting by a female rabbit can induce ovulation.[24] In cats, stimulation of the vagina will result in ovulation.[15,62] In ferrets, both vaginocervical stimulation and neck-gripping are necessary to induce ovulation.[9] Because of this elaborate procedure, it is not practical for owners to try to induce ovulation in jills. As an alternative, vasectomized hobs are used in the UK to induce ovulation.[32,60] One mating leads to cessation of estrus in about 75% of ferrets and two matings to cessation of estrus in 85% of ferrets.[60]

Effect: Termination of estrus followed by pseudopregnancy lasting about 42 days.[19]

Remarks: During pseudopregnancy, jills may display nesting behavior and enlargement of the abdomen and mammary glands.[19] The nesting behavior, which includes dragging cage mates around the cage and increased aggression towards the owners, does not make this an attractive option.

Vasectomized hobs remain aggressive and have a musky odor similar to that of intact hobs.

hCG or GnRH administration

Principle: After mating there is a preovulatory LH surge that may last to up to 12 hours.[12] This LH surge can be mimicked by the administration of either human chorionic gonadotropin (hCG) or indirectly by stimulating endogenous LH release with the hypothalamic releasing hormone GnRH.

Method: Ten days after the onset of estrus, 20 µg GnRH or 100 IU hCG is given intramuscularly.[19]

Effect: Approximately 35 hours after injection the ferrets ovulate, resulting in the formation of *corpora lutea* in 95% of the cases.[45] Vulvar swelling will start to decrease within 1 week of injection. Anoestrus (pseudopregnancy) will last for 40 – 60 days.[8]

Remarks: Fox *et al*. found that multiple injections of GnRH may sensitize the ferret to the drug, resulting in anaphylactic reactions shortly after administration.[19] Antihistamine administration ameliorates these reactions within minutes. The consequences of pseudopregnancy have been mentioned in the previous section and neutering ferrets with hCG or GnRH injections only applies to jills.

Manipulation of photoperiod and administration of melatonin

Principle: As described above, the ferret's reproductive season starts when there is more than 12 hours of light per day. During the scotophase, melatonin concentrations in plasma are high.[7,59] It has been speculated that keeping ferrets either under conditions with short photoperiods or giving them melatonin would suppress the pituitary-gonadal axis.

Method: Provision of a maximum of 8 hours of light per day, or daily administration of 1 mg melatonin 8 hours after the onset of light.[13]

Effect: Ferrets kept under 8h light : 16 h darkness (8L : 16D) come into estrus only 7 weeks later than ferrets exposed to long photoperiods (14L : 10D). When ferrets kept under long photoperiods (14L : 10D) received melatonin (1 mg/day) 8 hours after the onset of light, they come into estrus only 7 weeks later than ferrets kept under similar conditions and receiving oil injections.[13] Herbert *et al*. found that in the first year after ferrets were blinded they came into estrus at the expected time, but thereafter estrus synchrony was lost.[28] Estrus periods in blinded ferrets lasted from just a few weeks to up to 60 weeks.

Remarks: A limited light regimen and administration of melatonin are not effective in inhibiting the hypothalamus-pituitary-gonadal axis in ferrets.

Immunization against GnRH

Principle: There are several reports on the use of GnRH vaccines in mammals.[21,37,46,73] Depending on the species, these vaccines aim at contraception, the prevention of boar taint, or the control of hormone-dependent cancers, including breast and prostate cancers. In ferrets, use of a GnRH vaccine would serve two goals: inactive gonads in both sexes in combination with low plasma concentrations of gonadotropic hormones.

Method: Depending on the study inoculations were performed intramuscularly, subcutaneously or intranasally.

Effect: Decreased plasma gonadotropic hormone concentrations have been reported in male rats after immunization against GnRH.[37] Testosterone concentrations decreased in bulls, boars, male rats and dogs after immunisation,[21,37,46] while in female mice a sterilizing effect was seen.[73] Reduced testes size has also been reported in boars and rats after immunization against GnRH.[37,46]

Remarks: In a pilot study conducted at Utrecht University, eight out of twelve ferrets immunized against GnRH had to be euthanased (Schoemaker and others, in preparation). Post mortem examination disclosed aspecific lympho-plasmacellular infiltrations in multiple organs (liver, kidney, lung and intestines) suggesting an a-specific immune reaction. The background of these reactions has to be unraveled before GnRH immunization can be employed in ferrets.

Immunization with LH

Principle: Inhibition of LH secretion by immunization with heterologous LH.[18,34,55]

Method: Heterologous LH is injected intramuscularly or subcutaneously.

Effect: Injection of bovine LH causes a 90% reduction in the weight of rabbit testes, and genital atrophy in female rabbits and loss of receptiveness to males.[18] In another study with male rabbits, LH and testosterone plasma concentrations decreased significantly after immunization against LH; however, FSH concentrations increased significantly.[34] Similar, but less consistent, effects of LH immunization were seen in dogs.[42] In ewes estrus and pregnancy were prevented for 2 years after immunization against LH, although plasma LH concentrations were not lower than in control ewes.[55] In these ewes FSH concentrations were also increased.

Remarks: So far, there are no reports of LH vaccination in ferrets. Since not only LH but also FSH may influence the development of hyperadrenocorticism in ferrets, there is reason for caution with LH immunization in ferrets.

Immunization with LH receptor

Principle: Induction of LH receptor dysfunction by immunization with heterologous LH receptor.

Method: Immunization of bitches with 0.5 mg bovine LH receptor (bLH-R) encapsulated in a silastic subdermal implant, followed by intramuscular booster injections.[61]

Effect: In bitches immunization with bLH-R suppresses serum progesterone concentrations for approximately 1 year, while serum concentrations of oestradiol and LH are not affected. Although stimulation with GnRH in immunized dogs leads to a LH surge, serum progesterone concentrations do not increase. Thus bLH-R immunized bitches do not ovulate or do not produce active *corpora lutea*.[61]

Remarks: The main drawback of this approach is that ferrets may fail to ovulate. Prolonged estrus can be expected, which may result in bone-marrow suppression. In male mice, immunization against LH-R caused reduces androgen production.[54] In male ferrets, this might result in reduced aggressive behavior and decreased intensity of their musky

odor. Again some caution is warranted because high LH-R antibody titers in mice had an agonistic effect, resulting in hypertestosteronemia.[54]

Depot GnRH agonist

Principle: Depot GnRH agonists increase the levels of gonadotropic hormones, followed by a desensitization of gonadotroph receptors, resulting in decreased LH and FSH plasma concentrations.[69] The exact mechanism of the desensitization is still not clear.[30]

Method: Of the available formulations, leuprolide acetate (Lupron Depot 3.75 mg, TAP Pharmaceuticals Inc) is used to treat hyperadrenocorticism in ferrets.[70] Ferrets weighing less than 1 kg receive an intramuscular dose of 100 µg at monthly intervals and ferrets heavier than 1 kg receive 200 µg per month. A similar treatment protocol might also be effective for contraceptive purposes. Slow-release implants have been described in humans [14] and dogs.[68,69]

Effect: When leuprolide acetate is given to jills in estrus, ovulation will probably occur due to the initial LH surge seen after injection. Therefore leuprolide acetate should be administered before the breeding season, otherwise it has no advantage over regular GnRH. In dogs, however, leuprolide acetate induces estrus.[31] It is therefore uncertain whether this drug will be useful in neutering ferrets.

Remarks: Slow-release implants suppress reproductive function in dogs.[68] GnRH implants might therefore be an option for use in ferrets.

GnRH receptor antagonist

Principle: Competitive GnRH receptor occupancy with GnRH receptor antagonists results in a decreased release of gonadotropic hormones by the pituitary gland.[30]

Method: The available GnRH receptor antagonists have to be injected, but orally active non-peptide GnRH antagonists are currently being developed for use in humans.[48]

Effect: The initial increase in gonadotropic hormones, seen with GnRH agonists, are not seen with GnRH receptor antagonists. The use of these receptor antagonists will therefore result in an immediate decrease in gonadotrophic hormone concentrations.

Remarks: Until now, only a few GnRH receptor antagonists have been registered for use in humans.[30] New and longer-acting drugs are being developed. Degarelix (FE200486, Ferring) currently seems to be the most promising of these GnRH receptor antagonists.[11] While older GnRH antagonists caused increased histamine release after injection, the newer drugs do not have this side-effect. Nevertheless, local reactions at the site of injection are still common.[30] These drugs seem to be promising for future use in ferrets.

Conclusion

There are several potential ways to influence reproductive function in ferrets, other than surgical castration. Progestagens seem practical for use in jills but need to be studied for use in hobs. Possible future alternatives may be the use of slow-release GnRH implants, GnRH antagonists, or immunization against GnRH. Detailed studies are needed before these techniques can be recommended for neutering ferrets.

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