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Comparison of Gene-based and Hormonal Contraception Methods for Female Domestic Cats (*Felis silvestris catus*)

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Abstract. with the growing population of stray and feral cats, traditional contraception methods like spaying and neutering, though effective, present limitations for Trap-Neuter-Return (TNR) programs in terms of cost, feasibility, and health risks. This article compares and contrasts gene-based and hormonal contraception methods in female domestic cats (*Felis silvestris catus*). The article delves into alternative non-invasive methods, particularly focusing on AAV9-fcMISv2 gene therapy and various hormonal methods such as Megestrol Acetate, GnRH agonists, and melatonin, and reviewing the advantages and drawbacks of these methods. The comparative analysis highlights the promising potential of gene-based therapies as a long-term solution for controlling stray and feral cat populations.

Keywords: stray cats, gene-based contraception, hormonal contraception, AAV9-fcMISv2, animal welfare.

1. Introduction

1.1. Research background

The free-roaming stray and feral cat population continues to increase due to a lack of spaying and neutering, leading to uncontrolled reproduction. One pair of cats and their offspring can produce thousands of kittens in just a few years. To promote animal welfare, spaying or neutering has become a more humane approach to controlling stray cat populations compared to euthanizing individual cats. These methods can effectively reduce the number of kittens born into harsh conditions, preventing them from being threatened by extreme weather, starvation, and disease. Currently, the most popular and viable way to spay or neuter a cat is through ovariohysterectomy or ovariectomy, involving the removal of the reproductive tract, ovaries, and uterus. However, with the development of technology, some contraception methods, including hormonal and genetic contraception, have gradually stood out because of their non-invasive characteristics and suitability for large-scale application. This article reviews recent studies regarding these two innovative contraception methods and compares and contrasts their mechanisms, benefits, and drawbacks to explain why gene-based contraception has the highest potential in controlling the populations of stray and feral cats in the future.

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1.2. Research significance

This study deeply analyses the advantages and limitations of different non-invasive contraception methods, offering both theoretical and practical significance.

In terms of theoretical significance, this article highlights the cutting-edge techniques of non-invasive contraception methods and identifies research gaps in genetic studies and veterinary medicine, guiding future research to focus on key issues.

In terms of practical application, firstly, the comparative analysis results can help promote the development of cost-effective ways of neutering and spaying cats, providing support for the formulation of effective strategies to manage and control stray and feral cat populations. Secondly, this article contributes to the improvement of animal welfare. Promoting efficient non-invasive contraception methods can prevent the birth of kittens under harsh conditions and minimize the need for euthanasia. Additionally, this comparative analysis results in broader ecological and community health benefits, because neutering stray cats not only benefits the ecosystem by reducing cats' predation on wildlife but also reduces the noise and territorial fights during estrus as well as the spread of diseases when cats gather, helping to create a healthier and more secure community environment.

2. Gene-based Contraception Method: AAV9-fcMISv2

The now widespread gene-based therapy is AAV9-fcMISv2. By its name, AAV refers to the adeno-associated virus. Through single intramuscular injection, it introduces a purified and enzymatically cleaved FLAG-fcMISv2 into the host cells, which leads to elevated level of Anti-Müllerian hormone, reduced level of P4 (progesterone), and increased level of LH (Luteinizing Hormone), inhibiting the activation of primordial follicles and the complete maturation of ovarian follicles (Vansandt et al., 2023) [1]. The varied concentration of the above sexual hormones also decreases the frequency of the luteal phase, in which P4 causes the thickening of the uterine lining for the implantation of a fertilized egg. Furthermore, a situation similar to humans happens, in which higher levels of AMH, often seen in PCOS patients, might cause women to have difficulty releasing an egg, thus leading to infertility (Costa-Bir, 2021) [2].

In Vansandt et al. (2023) [1]'s experiment with nine domestic cats, none of the cats in the treated group got pregnant, while all of the cats in the control groups got pregnant and gave birth to an average of 10.5 kittens. Therefore, the method promises a 2-year duration of sterilization with a success rate of 100%. Besides its effectiveness, none of the cats showed abnormal signs of adverse effects despite trivial edema, and the modified individuals who maintain a high level of AMH even exhibit a lower chance of having cystic endometrial hyperplasia, a common threat to the population health of stray or feral cats.

Nonetheless, this contraception method has several limitations. One prominent drawback of using the AMH sequence is that prolonged MIS treatment was found to induce a menopausal-like state with disrupted estrus cycling and elevated gonadotropin levels. This requires hormone replacement therapy for long-term use. Moreover, the MIS protein might be expressed in various ways, resulting in inappropriate products with different levels of activity. Additionally, some stray or feral cats that are infected with feline panleukopenia virus (FPV) might have FPV antibodies, which might cross-react with closely linked virus species including AAV9. In fact, such prevalence of anti-AAV immunity including AAV2, AAV6, and AAV9 has been reported in the Northeastern United States (Adachi1 et al., 2020) [3]. Though plenty of Nabs lack neutralizing ability, there is still a possibility that AAV vectors end up binding with antibodies before entering host cells, increasing the administration difficulty.

3. Hormonal Contraception Methods

3.1. Megestrol Acetate

Megestrol Acetate (MA) is a commercially available progestin contraceptive that can either alter the motility of the tubular tract, resulting in failed oocyte transport and fertilization; or alter endometrium receptivity, resulting in failed implantation of fertilized eggs to the uterus lining (Vasetska, 2020) [4].

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Though its specific mechanism has not yet been discovered, it has been used to stop ovulation and follicular development in stray or feral cat populations in Europe and the U.S.

However, what is problematic is that its efficacy is guaranteed only if the treated female cats are separated from males for at least 1 week, which is hard to manipulate if female cats are released after the administration in real-life TNR cases. Additionally, the duration of effect ranges from 2-18 months, and referring to the experiment done by (Romatowski, 1989) [4], most MA-treated cats return to estrus within a few days. Moreover, MA can cause several side effects, including adrenocortical suppression, diabetes mellitus, endometrial hyperplasia (EH), pyometra, fibroadenomatous mammary hyperplasia, and mammary carcinomas (Greenberg et al., 2013) [5].

3.2. GnRH agonist

Gonadotropin-releasing hormone (GnRH) is released by the hypothalamus and is responsible for the secretion of follicle-stimulating hormone (FSH) and LH (Das, 2021) [6]. By utilizing the mechanism of negative feedback, GnRH agonists, peptides similar to GnRH, can be produced to inhibit GnRH action by binding to the GnRH-R, which further suppresses FSH and LH levels, making it to be below the threshold of follicular development, and subsequently disrupt luteal function and pregnancy. Long-term administration of GnRH agonists can also impair the positive feedback of estrogen, a vital sexual hormone that regulates female reproductive organs (Delgado & Lopez-Ojeda, 2023) [7], thus interrupting with menstrual cycle and preventing ovulation. GnRH can be applied through depot formulations or nasal sprays, which are therefore suitable to be applied to a large population. Furthermore, being able to manipulate ovarian function, GnRH agonists reduce the risk of feline diseases like breast cancer and uterine fibroids, improving the overall health of female domestic cats (Fraser, 1993) [8].

Even with its beneficial effects, GnRH had not been approved as a contraceptive because its suppression of estrogen can lead to hypo-estrogenic effects and may result in bone demineralization. In addition, the duration of its functions varies among individuals and application methods: nasal sprays require daily administration, while depot injection provides a longer period of 1-3 months. might cause unforeseen re-occurrence of sexual behaviors, so it requires frequent high-dose injections (Fraser, 1993) [8].

3.3. Melatonin

Melatonin is a naturally produced hormone primarily synthesized and secreted in the pineal gland in response to darkness. It is known for regulating circadian rhythms, while in fact, it can also be used as a contraceptive to suppress the reproduction cycle of female domestic cats. As the melatonin is administered orally or through implants, more melatonin reaches the hypothalamus, it inactivates the hypothalamic GnRH pulse generator, which sends fewer GnRH to the pituitary (Silman, 1993) [9]. The pituitary, in turn, would send fewer FSH and LH to the ovary, preventing the formation of follicles. Faya et al. (2011) [10] reported a success rate of 88.2% and 91.6% of non-ovulatory melatonin-treated cats showing a positive response to melatonin, defined as an interestrus interval longer than twice the control duration, with no signs of short-term physical side effects.

Nevertheless, according to the experiment, there is still a high proportion of the interestus intervals (43.3% and 36.8%) suggested signs of ovulation and pseudopregnancy compared to the non-ovulatory groups. The function of melatonin is limited to short-term modification of the reproduction cycle since the interestrus intervals are only prolonged by about 44-51 days compared to controls and all the treated cats are able to become pregnant and breed after the trials. Furthermore, this method is limited to post-puberal cats, since it did not significantly delay the onset of puberty in pre-pubertal cats in the experiment (Faya et al, 2011) [10].

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4. Discussion

The four contraception methods mentioned above can be compared and contrasted based on various aspects including their mechanisms, application methods, success rates, duration of effects, and adverse effects. The comparison is listed in the following table.

Table 1. Comparison of gene-based and hormonal contraception methods

	Mechanism	Administration method	Success rate (%)	Duration of effect	Adverse effects
AAV9-fcMISv2	By increasing AMH level and thus suppressing ovarian follicle development and ovulation	Intramuscular injection	100	2 years	No
Megestrol Acetate	By preventing the ovulation and follicular development	Oral administration or injection	No report	2-18 months	Adrenocortical suppression, diabetes mellitus, endometrial hyperplasia (EH), pyometra, fibroadenomato-us mammary hyperplasia, and mammary carcinomas
GnRH agonist implant	By blocking GnRH secretion and thus preventing the formation of follicles	Nasal sprays or depot injection		A maximum of 3 months	ypo-estrogenic effects and bone demineralization
Melatonin	By blocking GnRH secretion and thus preventing the formation of follicles	Implant or oral administration	88.2-91.6	44-51 days	No

Though via different pathways, the four contraception methods discussed share a similar mechanism that prevents ovulation and follicle formation. Besides, they can all be administered through non-invasive approaches like injections, oral administration, and nasal sprays that can be easily applied to a large number of individuals. Among them, AAV9- fcMISv2 gene-based therapy stands out as the potentially most effective, particularly for TNR groups that seek permanent contraception of stray or feral cats due to its ability to interfere with the reproductive process at the genetic level. It promises a success rate of 100% in recent clinical tests and lasts the most time compared with other contraception methods, with no prominent side effects that pose health issues to domestic cats. Relevantly, this gene modification will not affect the gene pool of other individuals, which is a common concern related to gene-edited organisms (GMOs), as sterilized female cats will not produce any offspring. However, it is still experimental and needs further research, so it is not a practical option for widespread use. Based on the table, hormonal methods, such as the use of megestrol acetate (MA), GnRH agonists, and melatonin, are also viable but are limited by their temporary nature and the potential for adverse health effects.

5. Conclusion

In conclusion, managing the stray or feral cat population through effective contraception is essential for promoting animal welfare, improving the community environment, and maintaining ecological balance.

Traditional contraception methods like spaying or neutering surgeries have long been prevalent, but their relatively high expense, high risk, and low feasibility for large populations call for the development of non-invasive alternatives. Among the contraception methods discussed, AAV9- fcMISv2 gene-based contraception is currently the best non-surgical option for sterilization because it can be its simple administration methods, high success rate, long duration of effects, and absence of side effects. Overall, in the future, the continued development of gene-based contraception methods will promise a humane and long-term solution to the overpopulation of stray or feral cats and play vital roles in TNR programs.

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