Research Report

Preliminary evaluation of a commercially available GnRH-based immunocontraceptive

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Abstract

Stray dog population control is a global concern due to its implications for public health and animal welfare. Immunocastration relies on the immunological blockade of gonadotropin releasing hormone (GnRH) and represents a viable alternative to surgical sterilization. We evaluated the effects of a commercially available immunocontraceptive on ovarian activity in prepubertal female dogs; 4 were treated with antiGnRH (2 treatments, 4 weeks apart) and 3 served as controls. Dogs were monitored for signs of estrus for 7-8 months after the second treatment and progesterone concentrations were determined. Ovaries and uterus were collected after ovariohysterectomy. None of the dogs had lesions or discomfort related to treatment; 3 out of 4 treated dogs did not have estrus until surgery (13-14 months of age) and 1 had estrus at 13 months; this dog had a corpus luteum and high progesterone concentrations. Ovaries were larger and had more follicles in treated dogs whereas endometrium was thicker in control. Commercially available antiGnRH can be a tool for reproductive management in prepubertal female dogs; however, further research is needed to confirm the long-term safety and duration of the contraceptive effect.

Keywords: GnRH, immunocontraceptive, ovary, follicle, pregnancy

Introduction

Uncontrolled canine reproduction remains a recurring challenge, with substantial consequences for public health and sanitary management. Reducing overpopulation and preventing zoonotic diseases are hard to achieve through the use of contraceptive methods in dogs and cats.[1] Surgical castration is currently the most common and permanent method whereas hormonal injections temporarily suppress the reproductive cycle but may induce adverse effects.[2]

Despite its effectiveness, surgical sterilization is often impractical for population-control programs due to its cost, requirement for surgical infrastructure and postoperative care. In a Brazilian free-roaming dog study, population size often did not decrease despite large sterilization efforts, mainly due to ongoing abandonment and migration.[1] Progestogens (e.g. progesterone) are not recommended because they may cause cystic endometrial hyperplasia, pyometra and increase the risk of mammary tumors.[2,3] These limitations have led to growing interest in nonsurgical, less invasive, and lower-cost methods, including immunocontraception that targets the gonadotropin releasing hormone (GnRH), which regulates the reproductive axis.[4]

GnRH is a peptide produced by the hypothalamus and released in pulses through the hypothalamic-pituitary system. It binds to specific receptors on pituitary gonadotrophs, stimulating the production and secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH). These gonadotropins have key roles in the development of the reproductive organs, gametogenesis and the synthesis of gonadal steroid hormones.[5]

Because GnRH is a small and highly conserved protein, it has been used as an immunogenic target in several species, including pigs,[6] goats,[7] cats,[8] rats[9] and across species.[8,10] Immunization with antiGnRH vaccines stimulates the immune system to produce antibodies that neutralize endogenous GnRH, blocking the release of LH and FSH and consequently suppressing reproductive function.[11] This inhibition also leads to gonadal atrophy due to reduced hormonal stimulation.[4]

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Immunocontraceptive method affects ovarian function (follicular maturation and ovulation) and consequently synthesis of sex hormones. Reduced circulating LH and FSH concentrations results in variations in progesterone and estradiol concentrations that in turn limit ovarian and uterine development and compromise reproductive organ maturation.[12,13]

Research on reproductive physiology and biotechnologies in dogs is essential to develop safer, more accessible contraceptive strategies. The study of nonsurgical, effective and reversible methods can benefit population-control programs, public institutions and nongovernmental organizations. Several studies have attempted to immunocastrate dogs with anti-GnRH vaccines;[12,14] however, to the best of our knowledge, only 2 studies used commercially available vaccines in dogs and fertility was not investigated.[4,11] Our objective was to evaluate the effects of a commercially available immunocontraceptive formulated for pigs on the ovarian activity of prepubertal female dogs. Our hypothesis was commercial antiGnRH vaccine temporarily blocks puberty in female dogs.

Materials and methods

This research was approved by the Animal Use Ethics Committee of Vila Velha University (CEUA–UVV, protocol number 707-2024). Seven medium-sized, mixed-breed intact female dogs aged 20-24 weeks, weighing 5-10 kg and with body condition scores between 4 and 5 on a 9-point scale,[15] were selected. Dogs had not yet experienced their first estrus and had never received hormonal contraceptives. Dogs B, C and D and dogs A, F and G were littermates; dogs A, B, and G served as controls and dogs C, D, E, and F were treated.

To stimulate antiGnRH antibodies production, dogs received GnRH-immunocontraceptive (Vivax, Zoetis, São Paulo, Brazil), a commercial vaccine developed for pigs. The product contains a synthetic GnRH analog conjugated to a carrier protein that induces antibody production against GnRH, using diphtheria toxoid as an immunostimulatory agent. GnRH-immunocontraceptive was given subcutaneously in the lateral thigh region at a dose of 0.25 ml, corresponding to 12.5% of recommended dose for pigs (2 ml). Dosage selected was based on a previous study in Wistar rats,[16] which demonstrated that this volume was safe and effective for inhibiting ovulation and pregnancy. As per manufacturer's recommendations for pigs, a booster injection (similar volume) was given after 4 weeks.

Throughout the study, animals were monitored for local reactions, fever, changes in appetite, pain or other clinical signs. Control dogs received an equal volume of sterile saline solution (0.9% NaCl) subcutaneously in the same anatomical region and at the same interval. Dogs were also observed by their owners for signs of estrus (vulvar swelling, attraction to male dogs, hyperemic vaginal mucosa and sanguineous vulvar discharge).

Dogs were housed in a fenced outdoor area, allowed free movement and fed a commercial diet based on body weight, with ad libitum access to water. Dogs had controlled contact with intact male dogs. Serum progesterone was measured using the cProg – Canine Progesterone Rapid Quantitative Test (Wondfo). Dogs B, C, D and E had progesterone measured at 3 time points, 30 days apart, starting at 11 months and ending on the day of ovariohysterectomy (OHE) for dogs that did not ovulate and at 16 months for the dog that did ovulate (we decided to delay OHE for this dog to avoid pseudocyesis). Dogs A and F had progesterone measured only at OHE due to logistical constraints. Dog G, a control animal, accidentally became pregnant; this dog was neither spayed nor was blood withdrawn for progesterone.

OHE was performed at ~ 13-16 months; this timing was chosen to avoid pseudocyesis in animals that ovulated. Before surgery, dogs had physical examination, complete blood count and biochemistry panel that included urea, creatinine, alanine aminotransferase, alkaline phosphatase, protein and fractions. Dogs were within normal parameters for surgery (conducted at the Veterinary Hospital of VVU under balanced inhalation anesthesia and standard aseptic technique). Each surgical team consisted of a surgeon, assistants and an anesthetist. Postoperative care continued until suture removal.

Collected ovaries and uteruses were fixed in 4% formalin and submitted to the UVV histopathology laboratory. Histological slides of each ovary and uterine tissue from each side were prepared for each animal, with 3 sections per slide, and stained with hematoxylin and eosin. Longitudinal sections encompassed the germinal region whereas transverse sections were made through the medial portion of uterine horns.

Because ovarian measurements under light microscopy at 4 x magnification were not feasible, slides were photographed with a semi-professional camera at a distance of 10.5 cm. Images were analyzed using ImageJ software, employing the 'freehand' tool to determine ovarian and uterine horn area, and the 'straight line' tool to measure endometrial thickness (mm). The number of follicles and corpora lutea were then evaluated under light microscopy at 4 x magnification. Only antral follicles with an oocyte were counted.

The number of animals that had no estrus, progesterone concentrations, number of follicles and number of CL are presented as numerical data. The size of the ovaries and endometrial thickness are presented as median and interquartile range (Q1-Q3). Analyses were performed using SAS 9.2 (North Carolina, SAS Institute Inc).

Results

Throughout the study, no differences were observed between groups in body weight or behavior. No swelling, redness, inflammation, or pain was identified in treated animals. Control dogs exhibited normal signs of estrus at ~ 8-10 months whereas 3 of 4 treated dogs had no estrous behavior until OHE (13-14 months). A treated dog (dog D) had vulvar swelling, hyperemic vaginal mucosa, sanguineous vulvar discharge at 13 months and was receptive to a male dog. A control dog (dog G) became pregnant at 10 months and could not participate in the surgical component of the study.

Serum progesterone concentrations in treated dogs were below diestrus reference range (10-20 ng/ml); until 12, 13 and 14 months in 1, 2 and 1 dogs, respectively (Table 1).

The mean area of the right and left ovaries in the control group was smaller (34.2 mm² and 37.3 mm², respectively) compared

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to treated group (46.9 mm² and 50 mm², respectively). The mean endometrial thickness in control dogs (3.75 mm) was higher than treated animals (2.25 mm). Size of ovaries and thickness of endometrium are summarized (Table 2).

Data on antral follicles and corpora lutea are summarized (Table 3). Antral follicle count was numerically lower in control dogs compared to treated dogs. Representative ovarian light microscopic images are provided (Figure); 1 treated (dog D) and 1 control (dog B) had corpora lutea.

Treated dogs had numerically larger ovaries and higher number of antral follicles; however, had no estrous behavior and had decreased serum progesterone concentrations. Among treated dogs, 3 out of 4 remained acyclic for 32 weeks after the second antiGnRH treatment; furthermore, 1 treated dog had signs only at 13 months.

Discussion

Dogs in the present study were clinically healthy before and after treatment with no behavioral changes or adverse reactions to the product. Although other studies have reported local side effects (e.g. mild swelling and lameness of the limb) on injection site side;[14] such manifestations were not observed in this experiment, indicating good tolerance to this commercial antiGnRH.

In dogs, the first estrous cycle typically occurs between 6 and 12 months, depending on breed and size.[17] Control dogs in this study exhibited estrus within that range. Among treated dogs, 1 dog expressed estrus at 13 months and the other 3 had no estrus until OHE surgery (13-14 months). Although the follow up period (13-14 months) was insufficient to determine the full duration of suppression, the findings suggested that ovarian inhibition lasted up to 6 months after the second treatment.

Dogs and other species treated with antiGnRH had a significant reduction of ovarian size and follicular activity.[13,18] We speculated that after vaccination, our dogs had decreases in GnRH, followed by decreases in LH. It is known that GnRH stimulation or blockade generally influence LH secretion more than FSH.[10,19] In dogs, LH secretion is strongly driven by GnRH pulse frequency, producing a rapid and pronounced surge. FSH, however, is less dependent on GnRH because its secretion is also regulated by inhibin, activin and follistatin, resulting in a broader and more prolonged release pattern.[20]

Low-intensity GnRH pulses are sufficient to stimulate continuous FSH secretion and early follicular growth, explaining the larger size of the ovaries and the greater number of antral follicles in treated dogs. However, lack of high-amplitude GnRH pulses prevented the preovulatory LH surge, interrupting ovulation.[5]

Lower progesterone concentrations (< 10 ng/ml) indicated lack of ovulation. Large studies across breeds and body weights had very consistent estrus progesterone profiles, with high, sustained progesterone concentrations > 20-30 ng/ml in the early and middiestrus phase.[21,22] Depending on assay methods (RIA, CLIA, ELISA) progesterone concentrations can slightly vary with high correlation.[23] Even the dog that ovulated had concentrations < 10 ng/ml until 12 months of age. A treated dog whose progesterone was measured only once (at 14 months) had no signs of estrus until such time.

Canine endometrium is highly steroid-dependent. Thickness and glandularity change across the estrous cycle. Histomorphometry

Table 2. Size of ovaries and thickness of endometrium

Variable	Group	Mean	Median (Q1-Q3)
Right ovary (mm²)	Control	34.21	34.21 (30.00-38.41)
	Treated	46.95	43.84 (33.05-60.85)
Left ovary (mm²)	Control	47.31	47.31 (46.94-47.68)
	Treated	50.09	48.26 (40.36-59.82)
Endometrial thickness (mm)	Control	3.75	3.75 (3.50-4.00)
	Treated	2.25	2.25 (1.75-2.75)

Table 3. Mean number of antral follicles and corpora lutea (CL) observed in the right and left ovaries

Dog	Treatment	Follicle number	Cl number
A	Control	18	0
B	Control	7	2
C	Treated	4	0
D	Treated	22	2
E	Treated	40	0
F	Treated	26	0

Table 1. Estrus signs and serum progesterone concentrations in control and treated dogs

Dog	Group	First GnRH (months)	Second GnRH (months)	Estrus signs (months)	First P_4 ng/ml/months	Second P_4 ng/ml/months	Third P_4 ng/ml/months	OHE (months)
A	Control	x	x	8	5.9/13	-	-	13
B	Control	x	x	10	21.4/11	23.1/12	9.2/13	13
C	Treated	5	6	No sign	2.8/11	4.6/12	8.9/13	13
D	Treated	6	7	13	5.1/11	7.9/12	31.9/13	16
E	Treated	5	6	No sign	2.5/11	3.5/12	4.0/13	13
F	Treated	5	6	No sign	5.7/14	-	-	14
G	Control	x	x	10 (pregnant)	-	-	-	-

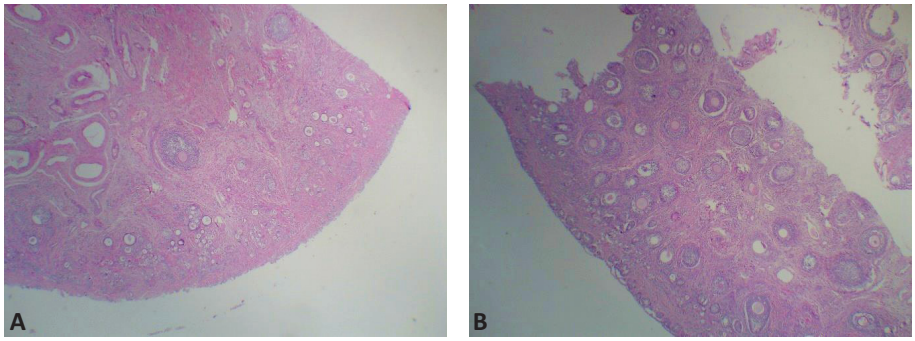

Figure. Light microscopic images (x 4) of ovaries from a control (A; dog B) and treated (B; dog E) dogs

identified progressive thickening occurring throughout the estrous cycle with maximal uterine wall and endometrial thickness in diestrus.[24] This remodeling is driven by estrogen and then progesterone. Estrogen promotes proliferative thickening and vascular changes whereas progesterone maintains a secretory, receptive state.[24,25] Treated dogs had decreased endometrial thickness, probably due to lack of progesterone exposure.

Most studies developed their own vaccines; a study conducted in Mexico used a commercial horse vaccine combined with a rabies vaccine to treat female dogs; antiGnRH antibodies and lack of progesterone were observed until day 61.[10] However, the main focus of the study was on vaccine safety. None of the studies using commercial vaccines evaluated fertility and reproductive parameters. A study that used a dog commercial vaccine measured testicular size but did not evaluate semen parameters and had a reversal of the vaccine effect on testicular size.[4] The duration of the immunocontraceptive effect remains a limitation to its use as a contraceptive.

Studies have demonstrated reduced concentrations of LH, FSH and testosterone in male mice treated with antiGnRH, with antibody titers declining by week 24 and a corresponding decreases in conception rates.[26] Beagle dogs had persistent antibody titers for up to 36 weeks[27] whereas 30% queens treated with a single dose of antiGnRH had contraceptive efficacy for at least 1 year.[8] These findings demonstrated that the effects of antiGnRH are temporary and new applications should be considered if continuous inhibition of cyclicity is desired. Dogs in the present study had no estrus for at least 6 months after the second antiGnRH treatment, demonstrating inhibition of estrus.

In male and female dogs,[14] these authors observed a partial reversal ~ on day 180, coinciding with a decline in antibody titers. Similarly, decreased immune response and contraceptive efficacy were reported after 20 weeks in housed dogs.[4] These results indicated that the success of immunocastration depended on maintaining sufficient antiGnRH antibody concentrations to block pituitary receptor binding. In other species, the duration of the effect varied: in domestic cats, 93% of infertility was observed during the first year, 73% after 2 years and 27% after 5 years.[28] Reduced follicle numbers and sizes with a predominance of atretic follicles were reported in gilts.[18] We observed an increase in follicle number and ovarian size, probably because of the immunogenic response that was more effective on LH secretion rather than on FSH as there was no ovulation in treated dogs.

Methodological constraints must also be acknowledged. Limitations include the small sample size that restricted data generalization, use of only prepubertal dogs, impeding the extrapolation to cycling dogs, and lack of antiGnRH antibody titration that would have allowed correlation between immune response and contraceptive effect magnitude. Furthermore, evaluation ended at OHE, precluding assessment of the full duration and reversibility of ovarian cyclicity.

Conclusion

This preliminary study demonstrated that antiGnRH treatment was well tolerated by dogs and reduced estrous behavior and ovulation up to 6 months after treatment. No adverse clinical or behavioral effects were observed, supporting the safety of the protocol. Overall, these findings suggested that a commercially available antiGnRH vaccine may be a safe, minimally invasive approach for temporarily controlling estrus. Further studies with larger cohorts and longer follow up are warranted.

Conflict of interest

None to report.

Authors' contribution and declaration

MR: collected data, involved in methodology, analyzed and interpreted data, and drafted the manuscript; **MF:** analyzed and interpreted data, supervised, reviewed, and edited and **BL:** conceptualized, designed the methodology, supervised the project, reviewed and edited. Authors have read and approved the final submission.

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