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Disappearance of signs of heat and induction of ovulation in oestrous queens with gonadorelin: a clinical study

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Abstract

Objectives The objective of this study was to assess the efficacy of a single intramuscular administration of gonadorelin to induce ovulation in queens in oestrus.

Methods Twenty-seven queens presented in oestrus for elective ovariectomy were divided into a treatment (n = 19) and a placebo (n = 8) group. Treated queens received a 50 µg dose of gonadorelin, while placebo-treated queens were injected intramuscularly (IM) with an equal amount of saline solution. All treatments were performed between the second and fourth days of heat.

Results Two days later, signs of behavioural heat had disappeared in all gonadorelin-treated queens, while 5/8 placebo-treated queens were still in heat. Following ovariectomy, performed 4 days after drug administration, the ovaries of each queen were evaluated histologically and the number of corpora lutea were counted. Sixteen of 19 (84%) gonadorelin-treated queens had ovulated and developed five (range 2–9) corpora lutea, while 3/8 (37%) placebo-treated queens had ovulated and developed five (range 3–6) corpora lutea.

Conclusions and relevance This is the first study to document the efficacy of a 50 µg/cat gonadorelin dose to induce ovulation in oestrous queens when administered IM on days 2–4 following the onset of oestrus.

Keywords: Ovulation; gonadorelin; corpora lutea; behavioural oestrus

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Introduction

Understanding feline ovulation has clinical and scientific relevance in veterinary practice.[1] In domestic queens ovulation normally follows a coitus-induced release of luteinising hormone (LH).[2,3] Ovulation generally occurs in a high percentage of queens mated more than once in early oestrus.[4] The release of LH in oestrous queens is followed by a quick disappearance of signs of heat due to ovulation and/or luteinisation of mature follicles followed by full development of corpora lutea (CL) and establishment of a luteal phase of 25–40 or 57–72 days' duration in non-pregnant and pregnant queens, respectively.[5–8] However, coital stimulation may sometimes be insufficient to achieve an LH peak of the necessary magnitude,[2,3] and failure to ovulate will result.[9]

The possibility of causing ovulation pharmacologically may be helpful in practice when dealing with infertile queens, to confirm diagnosis of ovarian remnant syndrome, to stop persistent heat in queens in which ovariectomy is not an immediate option, when planning an artificial insemination[5,10–16] or for dynamic testing in feline reproductive research.[17] Ovulation in queens can be achieved through mechanical stimulation of the

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vagina (using a glass rod or a cotton swab) or pharmacological administration of LH, LH-like drugs such as human chorionic gonadotropin (hCG) or LH-releasing compounds such as gonadotropin-releasing hormone (GnRH), also known as LH-releasing hormone (LH-RH), of which gonadorelin is a synthetic agonist.[18–23] The release of pituitary LH in queens may also occur spontaneously in females housed in cages or living separated from toms, as observed following human manipulation for veterinary procedures (blood collection) or exposure (without physical contact) to males.[24,25]

The two commercially available drugs most commonly used for ovulation induction in cats are hCG and gonadorelin. These two compounds are both effective in causing ovulation in oestrous queens, despite some important endocrine and molecular differences. From an endocrine point of view, GnRH mimics the hypothalamic hormone triggering the release of LH from the anterior pituitary causing secretion of ovarian progesterone, while hCG provides direct ovarian stimulation bypassing the hypothalamic–pituitary axis; this difference does not affect the efficacy of ovulation induction, but may be relevant in dynamic testing when pituitary responsiveness needs to be checked.

From a molecular point of view, hCG is a large glycoprotein hormone of molecular weight varying between 25 and 50 kDa, depending on the isoform.[26] Because of its oligosaccharide moieties, hCG has a long half-life and may remain in circulation for up to 4 days post-injection.[27] The consequent prolonged action often causes secondary follicular growth and accessory ovulations in treated cats – a fact that may alter the oviductal endocrine environment, thus lowering implantation rates in treated queens.[28] Also, the large hCG glycoprotein is capable of stimulating the immune system causing an immunological response when used frequently in reproductive control programmes. Swanson et al[29,30] have detected anti-hCG neutralising immunoglobulins in queens undergoing oestrus-induction protocols, which included hCG, with ovarian refractoriness occurring when queens were treated repeatedly every 50 days.

These considerations may be relevant in the choice of an ovulation induction strategy when applying oestrus-induction programmes in endangered feline species or whenever oestrus induction needs to be repeated over time. Because of its small size and simple molecule (nine amino acids, molecular weight 1.1–1.2 kDa), GnRH or its synthetic analogue gonadorelin do not cause ovarian hyperstimulation, and they are not detected by the immune system and therefore can be used repeatedly over time without the development of anti-GnRH antibodies.

Despite these immunological and half-life side effects, the use of hCG in cats has been the object of a vast body of literature over past decades,[31–33] while much less is known about GnRH as there are very few reports on its efficacy to induce ovulation in queens. When used as a single 25 µg/cat dose, gonadorelin caused ovulation in 4/4 cats treated on day 2 of oestrus.[23] The same dose administered intramuscularly (IM) to queens on days 2 or 3 of oestrus caused the development of a mean number of four CL and two anovulatory follicles in 10 queens;[34] however, no information was reported on whether any of the treated queens failed to ovulate, making calculation of percent efficacy of this treatment impossible.[34] The same 25 µg dose of gonadorelin/cat caused ovulation in 12/15 queens. Although the treatment was repeated twice at 12 h intervals, the number of CL was not reported and only 4/12 queens became pregnant.[34]

Because of insufficient information on the efficacy of the 25 µg/cat dose of gonadorelin, feline clinicians sometimes use higher GnRH doses in queens, such as 50 µg/cat. In our clinical practice we routinely and satisfactorily use gonadorelin as a single dose of 50 µg/cat IM to induce ovulation in oestrous queens or for dynamic testing in research protocols (unpublished observation). However, there is no information on the clinical efficacy of such a dose when administered to oestrus queens. The aim of the present study was to evaluate the efficacy of a single dose of 50 µg gonadorelin in causing ovulation in oestrous queens by evaluating the disappearance of oestrous behaviour after treatment and by histological demonstration of the presence and number of CL on ovaries removed by ovariectomy 4 days following drug administration.

Materials and methods

This study was conducted on privately owned queens presented for elective gonadectomy at the University of Pisa during the period 1996–1997. The Ethics Committee of the University of Pisa approved the study retrospectively (reference number 29/2020). Criteria for inclusion of queens in the study were to be in heat at the time of initial presentation, to have no history of reproductive or general diseases, and to be in normal general and reproductive health.

Upon initial presentation, the owners were questioned about their understanding of feline oestrous behaviour and the main signs of heat in the queen were quickly reviewed[6] in order to ensure a common knowledge base as a tool to monitor treatment efficacy. An informed consent form was signed by each owner, where it was clearly specified that their animals would be included in a clinical research programme in which a veterinary drug was being tested to suppress signs of heat, making it clear that the choice of whether to use the treatment or a placebo was random.

A complete physical examination was carried out for each queen to confirm the normality of health conditions and to rule out a history of any abnormality of general and reproductive health. Soundness of the reproductive system was confirmed through history, vaginal cytology

and transabdominal uterine palpation. Vaginal smears were performed with a cotton swab and stained with Diff-Quik (MGG Quick stain; Bio-Optica). Evaluation of vaginal smears was performed at ×4 under light microscopy (Olympus BX-40; Olympus) and the queens were classified according to the prevalence of the cellular type into either oestrus (>50% keratinised) or interoestrus–anoestrus (<50% keratinised).

Queens selected for the study were randomly divided into a treatment and a placebo group. Treated queens received a 50 µg dose (total volume 0.5 ml) of gonadorelin (Fertagyl; Intervet) IM, while placebo-treated queens were injected IM with an equivalent amount of saline solution (Fisiovet; Braun). Owners were requested to make sure their queens would not come into contact with any tom cat between administration of gonadorelin and ovariectomy, and to bring their animals back 4 days later for surgery.

On the day of surgery queens were given a thorough clinical examination to make sure their health conditions were still the same as previously and to check whether the drug administered had showed any effect on duration of oestrous behaviour, as well as any local reaction at the injection site. Ovariectomy was performed using a standard midline approach. Anaesthesia was performed by premedicating with 0.55–1.1 mg/kg xylazine (Rompun; Bayer) followed by 11–22 mg/kg ketamine (Ketaver; Gellini), inducing anaesthesia with 4 mg/kg intravenous (IV) propofol (Proposure; Codifa) and maintaining it with 1.5–2% isoflurane (Isoflo; Zoetis).

Immediately after ovariectomy, the ovaries were evaluated macroscopically in order to do a preliminary count of the number of CL and to make sure that both poles were intact and no ovarian remnant had been left attached to the caudal ovarian pedicle. Subsequently, ovaries were cut in half and fixed in a 10% neutral formalin solution (pH 7.4) for 48 h. After dehydration in a graded series of ethanol they were routinely processed and paraffin embedded. Four micrometre-thick sections were cut and stained with haematoxylin and eosin. Counting of CL[6] was performed under light microscopy at ×4.

Data relative to the disappearance of signs of heat and ovulation rate in treated vs placebo-treated queens were analysed using the χ^2 test, setting the significance threshold at $P < 0.05$.

Results

Twenty-seven queens (20 European, four Persian and three Siamese) were selected from those presented for elective ovariectomy; 19 and eight were assigned to the GnRH and placebo treatments, respectively. Queens included in the study were 21 months old (range 6–48 months) and weighted 3.1 kg (range 2.0–4.2 kg) (Table 1). On the day of initial presentation (day 0), 20/27 queens had been in heat for 2 days, 1/27 queens was in the third day, 5/27 queens were in their fourth day and 1/27 queens was in the fifteenth day of heat. Based on vaginal cytology, all queens were in full oestrus, with 80–100% of keratinised vaginal epithelial cells (Table 1). The interval between the initial presentation and the day of surgery was: (1) 3–4 days for 22/27 queens; (2) 2, 5 and 9 days for one queen each; and (3) 6 days for two queens (Table 1).

On the day of ovariectomy, all queens were reported to have been in a normal health condition since the day of treatment/placebo administration without any side effects at the injection site. Owners of GnRH-treated queens reported that behavioural oestrus disappeared in all queens during days 1 and 2 post-treatment, while owners of placebo-treated queens reported that during the same 2 days signs of oestrus disappeared in only 3/8 queens. Upon clinical examination there was a significant difference in the incidence of signs of behavioural heat between the 19 GnRH-treated queens (none of them was in heat and their vaginal smears were 60–70% non-keratinised), while 5/8 placebo-treated queens were still in behavioural heat (Table 1), with their smears still 80–100% keratinised ($P \leq 0.05$). Surgery was uneventful and all queens recovered well.

On histology, 84% (n = 16/19) of GnRH-treated queens had ovulated and developed five CL (range 2–9 CL), while the remaining three queens were not in heat but had not ovulated (Figure 1). Only 3/8 (37%) placebo-treated queens ovulated and developed five CL (range 3–6 CL) ($P \leq 0.05$) (Table 2). Three of eight placebo-treated queens that were still in heat had ovulated, while the remaining two had not ovulated and were not showing any signs of heat. When considering the day of heat in which queens were injected with GnRH, 13/15 (86.7%) queens treated on day 2 ovulated, while 4/5 (80%) queens treated on day 3 or 4 ovulated. However, this difference was not statistically significant.

Discussion

In the present study, a single dose of 50 µg of gonadorelin caused the disappearance of signs of heat and ovulation in 84% of treated queens with the development of five CL (range 2–9 CL), while only 37% of placebo-treated queens ovulated and produced five CL (range 3–6 CL). When considering the day of heat in which queens were treated in our study, earlier (day 2 of heat) treatments were associated with a very high (86.5%) ovulation rate. This fact is surprising when considering that failure to conceive in queens owing to failure to ovulate is reported to occur on day 1 or 2 of oestrus, although this refers to natural mating.[2–4,9]

Swanson et al[35] reported an 80% (12/15 queens) ovulation rate when treating queens on day 4 of oestrus with a 25 µg gonadorelin dose (albeit this dose was administered twice at 12 h intervals), which is in agreement with the 80% ovulation rate obtained in our queens treated on days 3–4 of heat. Goodrowe and Wildt reported an

Table 1 Breed, age, number of days in heat at first presentation, percentage of keratinised epithelial cells on the vaginal smear, date of initial presentation, treatment, date of ovariectomy, number of days between initial presentation and surgery, presence of signs of oestrus on the day of surgery and number of corpora lutea found at ovariectomy in 27 adult queens in heat treated intramuscularly with 50µg gonadorelin (Fertagyl; Intervet) or placebo

Cat number	Breed	Age (months)	Weight (kg)	Number of days in heat at first visit	% keratinised cells on vaginal smear at first visit	Date of first visit	Treatment	Date of ovariectomy	Days from first visit to surgery	Presence of behavioural heat on the day of surgery	Number of corpora lutea
1	Persian	7	3	2	97	05/01/96	GnRH	09/01/96	4	Absent	5
2	European	12	3.2	2	100	15/01/96	GnRH	19/01/96	4	Absent	6
3	European	10	2.5	2	93	19/01/96	Placebo	22/01/96	3	Present	0
4	European	8	2.6	4	88	25/01/96	GnRH	29/01/96	4	Absent	0
5	European	8	3	4	97	25/01/96	GnRH	29/01/96	4	Absent	3
6	European	8	2.5	2	100	29/02/96	GnRH	04/03/96	4	Absent	5
7	European	7	2	2	99	29/02/96	Placebo	04/03/96	4	Present	0
8	European	10	3.5	15	97	10/04/96	GnRH	12/04/96	2	Absent	3
9	European	8	2.5	2	90	16/04/96	GnRH	20/04/96	4	Absent	5
10	European	24	4	2	85	22/04/96	GnRH	26/04/96	4	Absent	6
11	European	8	3	2	96	02/05/96	GnRH	06/05/96	4	Absent	0
12	Siamese	7	3.2	2	75	09/05/96	Placebo	13/05/96	4	Present	3
13	European	18	4.2	3	98	10/05/96	GnRH	14/05/96	4	Absent	5
14	Persian	18	4.1	2	98	15/15/96	GnRH	21/05/96	6	Absent	8
15	European	10	3.5	2	98	10/06/96	GnRH	14/06/96	4	Absent	2
16	European	48	3.2	4	96	12/06/96	GnRH	18/06/96	6	Absent	3
17	European	10	3	4	94	14/06/96	GnRH	18/06/96	4	Absent	4
18	European	42	2.5	2	100	25/06/96	GnRH	29/06/96	4	Absent	4
19	Persian	48	4	2	99	25/06/96	Placebo	28/06/96	3	Absent	0
20	European	24	3.6	2	96	26/06/96	GnRH	29/06/96	3	Absent	2
21	European	25	3.7	2	96	01/07/96	Placebo	05/07/96	4	Present	6
22	Siamese	17	3.3	4	88	22/07/96	Placebo	26/07/96	4	Absent	0
23	Persian	12	3.1	2	99	30/09/96	GnRH	05/10/96	5	Absent	3
24	European	8	2.5	2	95	07/01/97	GnRH	10/01/97	3	Absent	9
25	Siamese	16	3	2	99	13/01/97	GnRH	17/01/97	4	Absent	0
26	European	19	4	2	80	21/01/97	Placebo	24/01/97	3	Present	5
27	European	6	2	2	88	17/03/97	Placebo	26/03/97	9	Absent	0

GnRH = gonadotropin-releasing hormone

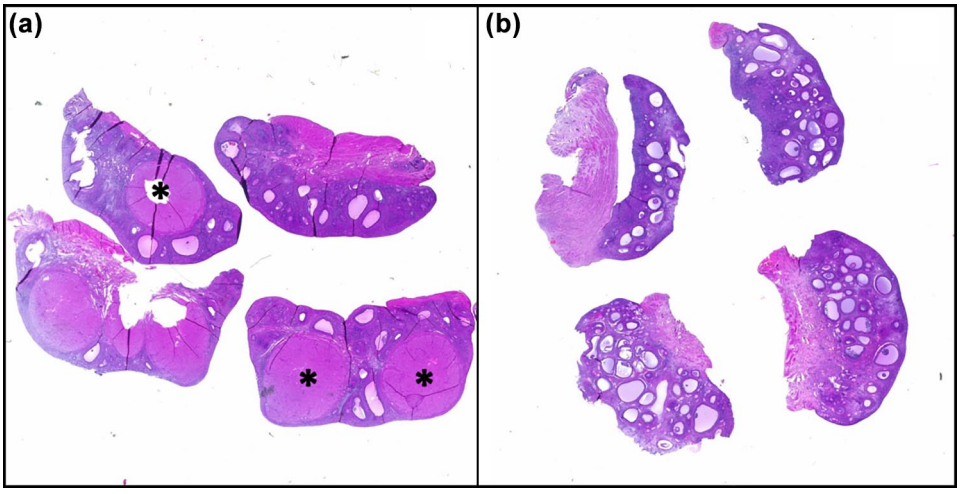

Figure 1 (a) Left (upper) and right (bottom) ovary of queen 12 treated intramuscularly (IM) with 50 µg gonadorelin gonadotropin-releasing hormone on day 2 of heat and ovariectomised 4 days later, presenting three corpora lutea (black asterisk). (b) Left (upper) and right (bottom) ovary of queen 19 treated IM with a placebo on day 2 of heat and ovariectomised 3 days later, presenting only primary and secondary follicles without any evidence of ovulation

Table 2 Incidence of ovulation with or without the presence of behavioural and vaginal cytology signs of heat in 27 queens treated intramuscularly with 50 µg/cat gonadorelin or a placebo when in heat

Results	Number of queens treated with GnRH	Number of queens treated with placebo
Ovulation, absence of signs of heat	16	0
Ovulation, presence of signs of heat	0	3
No ovulation, absence of signs of heat	3	3
No ovulation, presence of signs of heat	0	2
Total	19	8

Ovulations were determined by counting the number of corpora lutea at ovariectomy. The presence of at least one corpora lutea was considered as evidence that the queen ovulated
GnRH = gonadotropin-releasing hormone

average development of four CL/queen in 10 queens treated with 25 µg gonadorelin on days 2 and 3 of heat,[34] although they did not specify whether any of their queens failed to ovulate.

The interval between treatment and the disappearance of signs of heat was very short for treated cats, as in all 19 gonadorelin-treated queens heat disappeared within 48 h. However, 3/19 (16%) queens had no CL on their ovaries, indicating that the disappearance of signs of oestrus in these three cats was actually due to a natural transition into post-oestrus occurring at the time of treatment with a consequent failure to ovulate. The 16% incidence of failure to ovulate of our gonadorelin-treated cats is a slightly lower value when compared with the 20% rate in the only other study reporting on this aspect.[34] Failure of CL development in oestrous queens treated with GnRH may be due to a lack of pituitary responsiveness to GnRH. This may be the consequence of follicular atresia at the time of treatment reducing positive oestrogen feedback on the pituitary.[36] Follicular atresia at the time of GnRH treatment could indicate: (1) a very short heat; or (2) a lack of proper recognition of (perhaps mild) signs of oestrous behaviour by the owner, in which case the queen would be at a more advanced stage of oestrus at the time of initial presentation. Although owner compliance in this study was good, the latter hypothesis cannot be ruled out. This hypothesis is consistent with a seemingly higher ovulation rate in our queens when treatment was performed on day 2 of oestrus. The lack of statistical significance between the rates of ovulation on day 2 vs days 3–4 in the queens of our study might be due to a small sample size.

Three of eight placebo-treated queens ovulated following the administration of saline solution, indicating either spontaneous or potentially induced ovulation.[24,25] LH release occurring in these queens may have been elicited at home owing to: (1) viewing or being in proximity to a male cat; (2) during handling/petting procedures or at the veterinary clinic owing to (3) viewing or being in proximity to a male cat; (4) while inserting the vaginal swab to do vaginal cytology; or (5) the treatment being administered.[24,25] Items (1), (2) and (3) would indicate spontaneous ovulation. The 37% ovulation rate observed in our placebo-treated queens (five European, one Siamese and two Persian) is in agreement with the

35% incidence of non-coital ovulation observed in 7/20 experimental domestic shorthair queens kept in cages and handled only for blood collection.[24] Items (4) and (5) would indicate induced ovulation. Although induced ovulation cannot be ruled out, there is no scientific evidence that a single intramuscular injection or a single insertion of a vaginal swab may cause ovulation in oestrous queens.

This is the first study to investigate the effects of a 50 µg/cat intramuscular gonadorelin dose on inducing ovulation and to report on the interval between treatment and the disappearance of signs of oestrus in queens treated in early oestrus. The fact that our results are in agreement with those of Goodrowe and Wildt[34] and Swanson et al[35] indicates that a 25 µg/cat dose of GnRH might be as effective as a dose of 50 µg/cat to induce ovulation in queens treated between days 2 and 4 of heat. However, neither dose appears to be fully effective as the ovulation rate never reaches 100%.

Conclusions

Although lower doses of gonadorelin such as 25 µg/cat might be equally effective, more research is necessary to know the number of CL produced and the incidence of ovulatory failure with different doses of gonadorelin in the same sample of oestrous queens. GnRH could be a useful adjunct or even a better treatment than hCG to induce ovulation in cats, particularly when dealing with wild felids when there is a need to repeat oestrus induction strategies over time.

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Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval This work involved the use of non-experimental animals (owned or unowned) and procedures that differed from established internationally recognised high standards ('best practice') of veterinary clinical care *for the individual patient*. The study therefore had ethical approval from an established committee as stated in the manuscript.

Informed consent Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animals described in this work (either experimental or non-experimental animals) for the procedure(s) undertaken (either prospective or retrospective studies). No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

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