

Determining Whether a Dog is Spayed

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History

Queenie, a mature adult female boxer, was evaluated following adoption from a local shelter. The shelter presumed she had been spayed because of a scar on her ventral midline consistent with an ovariohysterectomy (OHE); however, within a month of adoption, Queenie developed vulvar swelling and began to attract male dogs.

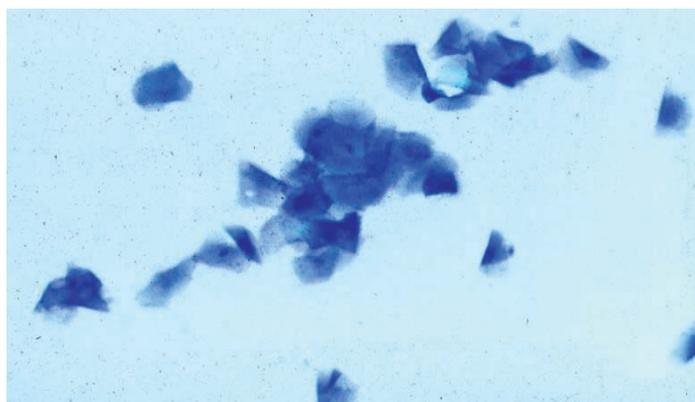
Examination

Queenie was bright and alert; had a body condition score of 5/9; and showed minor mammary gland development, moderate vulvar swelling, and slight serosanguinous vaginal discharge. The remainder of the examination was unremarkable.

Initial Diagnostic Evaluation

A vaginal cytology sample was collected to determine if the vulvar swelling and discharge were related to elevated estrogen levels. The cytology sample swab was rolled on the slide, air dried, and stained with Diff-Quik before being examined. The sample contained predominately large, cornified superficial cells with pyknotic nuclei and few other cells or background debris (**Figure 1**).

The presence of cornified epithelial cells confirmed estrogen stimulation. To determine if the estrogen exposure was endogenous or exogenous, the owner was thoroughly questioned about recent administration of oral or injectable estrogens and possible contact with human transdermal hormone replacement therapy (HRT). Dogs are very sensitive to HRT and can show signs of estrogen stimulation after contact with persons, clothing, or bedding contaminated with HRT.¹ Additional testing to confirm the presence of ovarian tissue was recommended.



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Vaginal cytology with large cornified epithelial cells or superficial cells indicating elevated estrogen concentrations.

Queenie, a mature female boxer, was presented after developing vulvar swelling and attracting male dogs.



Ask Yourself



What additional diagnostic tests can be performed to determine if this patient has ovarian tissue?

- A. Luteinizing hormone test
- B. Serum progesterone concentration
- C. Anti-Müllerian hormone assay
- D. Ultrasound
- E. All of the above

HRT = hormone replacement therapy,
OHE = ovariohysterectomy

continues

BEST ANSWER

E. All of the above

A combination of history, examination findings, and diagnostic tests are used to diagnose the presence of ovarian tissue. The same strategy can be used to determine if a bitch is intact, spayed, or has an ovarian remnant (OR).

Luteinizing hormone (LH) is negative (<1 ng/mL) in intact bitches or those with ovarian remnant syndrome (ORS). In addition, LH is negative in bitches exposed to endogenous or exogenous estrogen.² LH tests are not performed if the patient is currently showing signs of estrogen stimulation. A negative result in a nonestrogenized bitch is consistent with the presence of ovarian tissue.

Serum LH is positive (>1 ng/mL) in bitches without ovarian tissue unless the test has detected the LH surge for that cycle. The LH test can be used to screen for ovarian tissue if the bitch does not show evidence of estrogen stimulation at the time of evaluation. Two positive tests performed several days apart (to ensure the sample was not taken during the LH surge) provide conclusive evidence that ovarian tissue is not present. Major veterinary

Ovarian remnant syndrome is caused by incomplete or improper removal of the ovaries during OHE or OVE

laboratories perform LH tests, and an LH test kit is also available (Witness LH, zoetis.com) for in-house use.

Progesterone concentrations are consistently <0.2 ng/mL in bitches without ovarian tissue and rise following ovulation in intact bitches or those with ORS. Documenting serum progesterone >5 ng/mL in a bitch with evidence of estrogen stimulation 3–4 weeks prior confirms the presence of ovarian tissue. In addition, progesterone concentrations <1 ng/mL alone do not rule out ORS, as the dog may be in between heat cycles (anestrus). Quantitative progesterone measurements are performed at major veterinary laboratories.

Anti-Müllerian hormone (AMH) is produced solely by the ovaries in females,³ so AMH measurement can be used to distinguish between intact and spayed bitches. The AMH ELISA can be used successfully to diagnose ORS in bitches. The in-house lateral flow test is not recommended for ORS screening as it is not sensitive enough to detect some ORS cases.² AMH is not suppressed by estrogen and can be used at any time in post-pubertal bitches to evaluate for the presence of ovaries. The AMH ELISA is currently performed at the Animal Health Diagnostic Center at Cornell University; the Clinical Endocrinology Laboratory at University of California, Davis; and AViD Laboratories.

Although ovarian tissue can be seen on ultrasound, it is most successfully employed when the bitch is in estrus and has active follicular tissue and the ovaries are in their normal location.



Tips for Successful ORS Surgery

- Make a large incision to allow complete visualization of the abdomen.
- Thoroughly examine all mesenteric surfaces.
- Submit the excised tissue for histopathological evaluation.
- Perform the surgery 2–4 weeks after the bitch goes out of heat.
 - This is when the luteal tissue (progesterone-producing tissue) is prominent and the remnant is most easily located.

AMH = anti-Müllerian hormone, LH = luteinizing hormone, OHE = ovariectomy, OR = ovarian remnant, ORS = ovarian remnant syndrome, OVE = ovariectomy

Ovarian remnants can be located anywhere in the abdomen and can be challenging for the most experienced ultrasonographer to find, particularly if the tissue is inactive at the time of evaluation or in an unexpected location.

ORS is caused by incomplete or improper removal of the ovaries during OHE or ovariectomy (OVE).⁴ Ectopic ovarian tissue does not occur in the bitch. Surgeon experience is not correlated with ORS incidence. The right ovary is affected most frequently, and the interval from OHE/OVE to diagnosis is 1 month–10 years.³ Factors thought to increase risk for ORS include inadequate incision length, poor exposure, and failure to examine the ovaries after removal.³

Treatment

Exploratory laparotomy and excision of OR(s) is the treatment of choice. The majority of remnants are found in the region of the pedicles. A complete exploratory should be performed to ensure all ovarian tissue is removed. Ovarian remnants can also be created by fracturing of the ovary during OHE/OVE and subsequent revascularization of the ovarian tissue. These remnants can be located anywhere in the abdomen and can require a lengthy search to uncover. ■ **cb**

The Take-Home

- A combination of history, physical examination findings, and hormone testing is used to determine if a bitch is intact or has an ovarian remnant.
- Hormone testing choice and timing depends on presence of current estrogen stimulation and where the bitch may be in her cycle.

References

1. **Ongoing safety review of Evamist (estradiol transdermal spray) and unintended exposure of children and pets to topical estrogen.** FDA Drug Safety Communication; <http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm220185.htm>; accessed Jan 2015.
2. **Measurement of serum anti-Müllerian hormone concentration in female dogs and cats before and after ovariohysterectomy.** Place NJ, Hansen BS, Cheraskin JL, et al. *J Vet Diagn Invest* 23:524-527, 2011.
3. **Ovarian remnant syndrome in dogs and cats: 21 cases (2000-2007).** Ball RL, Birchard SJ, May LR, et al. 2010. *JAVMA* 236:548-553, 2010.
4. **Pituitary effects of steroid hormones on secretion of follicle-stimulating hormone and luteinizing hormone.** Nett TM, Turzillo AM, Baratta M, et al. *Domest Anim Endocrinol* 23:33-42, 2002.

NexGard® (afoxolaner) Chewables

CAUTION: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

Description:

NEXGARD® (afoxolaner) is available in four sizes of beef-flavored, soft chewables for oral administration to dogs and puppies according to their weight. Each chewable is formulated to provide a minimum afoxolaner dosage of 1.14 mg/lb (2.5 mg/kg). Afoxolaner has the chemical composition 1-Naphthalenecarboxamide, 4-[5-(3-chloro-5-(trifluoromethyl)-phenyl)-4,5-dihydro-5-(trifluoromethyl)-3-isoxazolyl]-N-[2-oxo-2-(2,2,2-trifluoroethyl)amino]ethyl.

Indications:

NEXGARD kills adult fleas and is indicated for the treatment and prevention of flea infestations (*Ctenocephalides felis*), and the treatment and control of Black-legged tick (*Ixodes scapularis*), American Dog tick (*Dermacentor variabilis*), and Lone Star tick (*Amblyomma americanum*) infestations in dogs and puppies 8 weeks of age and older, weighing 4 pounds of body weight or greater, for one month.

Dosage and Administration:

NEXGARD is given orally once a month, at the minimum dosage of 1.14 mg/lb (2.5 mg/kg).

Dosing Schedule:

Body Weight	Afoxolaner Per Chewable (mg)	Chewables Administered
4.0 to 10.0 lbs.	11.3	One
10.1 to 24.0 lbs.	28.3	One
24.1 to 60.0 lbs.	68	One
60.1 to 121.0 lbs.	136	One
Over 121.0 lbs.	Administer the appropriate combination of chewables	

NEXGARD can be administered with or without food. Care should be taken that the dog consumes the complete dose, and treated animals should be observed for a few minutes to ensure that part of the dose is not lost or refused. If it is suspected that any of the dose has been lost or if vomiting occurs within two hours of administration, redose with another full dose. If a dose is missed, administer NEXGARD and resume a monthly dosing schedule.

Flea Treatment and Prevention:

Treatment with NEXGARD may begin at any time of the year. In areas where fleas are common year-round, monthly treatment with NEXGARD should continue the entire year without interruption.

To minimize the likelihood of flea reinfestation, it is important to treat all animals within a household with an approved flea control product.

Tick Treatment and Control:

Treatment with NEXGARD may begin at any time of the year (see **Effectiveness**).

Contraindications:

There are no known contraindications for the use of NEXGARD.

Warnings:

Not for use in humans. Keep this and all drugs out of the reach of children. In case of accidental ingestion, contact a physician immediately.

Precautions:

The safe use of NEXGARD in breeding, pregnant or lactating dogs has not been evaluated. Use with caution in dogs with a history of seizures (see **Adverse Reactions**).

Adverse Reactions:

In a well-controlled US field study, which included a total of 333 households and 615 treated dogs (415 administered afoxolaner; 200 administered active control), no serious adverse reactions were observed with NEXGARD.

Over the 90-day study period, all observations of potential adverse reactions were recorded. The most frequent reactions reported at an incidence of > 1% within any of the three months of observations are presented in the following table. The most frequently reported adverse reaction was vomiting. The occurrence of vomiting was generally self-limiting and of short duration and tended to decrease with subsequent doses in both groups. Five treated dogs experienced anorexia during the study, and two of those dogs experienced anorexia with the first dose but not subsequent doses.

Table 1: Dogs With Adverse Reactions.

	Treatment Group			
	Afoxolaner		Oral active control	
	N ¹	% (n=415)	N ²	% (n=200)
Vomiting (with and without blood)	17	4.1	25	12.5
Dry/Flaky Skin	13	3.1	2	1.0
Diarrhea (with and without blood)	13	3.1	7	3.5
Lethargy	7	1.7	4	2.0
Anorexia	5	1.2	9	4.5

¹Number of dogs in the afoxolaner treatment group with the identified abnormality.

²Number of dogs in the control group with the identified abnormality.

In the US field study, one dog with a history of seizures experienced a seizure on the same day after receiving the first dose and on the same day after receiving the second dose of NEXGARD. This dog experienced a third seizure one week after receiving the third dose. The dog remained enrolled and completed the study. Another dog with a history of seizures had a seizure 19 days after the third dose of NEXGARD. The dog remained enrolled and completed the study. A third dog with a history of seizures received NEXGARD and experienced no seizures throughout the study.

To report suspected adverse events, for technical assistance or to obtain a copy of the MSDS, contact Merial at 1-888-637-4251 or www.merial.com/nexgard. For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or online at <http://www.fda.gov/AnimalVeterinary/SafetyHealth>.

Mode of Action:

Afoxolaner is a member of the isoxazoline family, shown to bind at a binding site to inhibit insect and acarine ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA), thereby blocking pre- and post-synaptic transfer of chloride ions across cell membranes. Prolonged afoxolaner-induced hyperexcitation results in uncontrolled activity of the central nervous system and death of insects and acarines. The selective toxicity of afoxolaner between insects and acarines and mammals may be inferred by the differential sensitivity of the insects and acarines' GABA receptors versus mammalian GABA receptors.

Effectiveness:

In a well-controlled laboratory study, NEXGARD began to kill fleas four hours after initial administration and demonstrated >99% effectiveness at eight hours. In a separate well-controlled laboratory study, NEXGARD demonstrated 100% effectiveness against adult fleas 24 hours post-infestation for 35 days, and was > 93% effective at 12 hours post-infestation through Day 21, and on Day 35. On Day 28, NEXGARD was 81.1% effective 12 hours post-infestation. Dogs in both the treated and control groups that were infested with fleas on Day 1 generated flea eggs at 12- and 24-hour posts-treatment (0-11 eggs and 1-17 eggs in the NEXGARD treated dogs, and 4-90 eggs and 0-118 eggs in the control dogs, at 12- and 24-hours, respectively). At subsequent evaluations post-infestation, fleas from dogs in the treated group were essentially unable to produce any eggs (0-1 eggs) while fleas from dogs in the control group continued to produce eggs (1-141 eggs).

In a 90-day US field study conducted in households with existing flea infestations of varying severity, the effectiveness of NEXGARD against fleas on the Day 30, 60 and 90 visits compared with baseline was 98.0%, 99.7%, and 99.9%, respectively. Collectively, the data from the three studies (two laboratory and one field) demonstrate that NEXGARD kills fleas before they can lay eggs, thus preventing subsequent flea infestations after the start of treatment of existing flea infestations.

In well-controlled laboratory studies, NEXGARD demonstrated >94% effectiveness against *Dermacentor variabilis* and *Ixodes scapularis*, 48 hours post-infestation, and against *Amblyomma americanum* 72 hours post-infestation, for 30 days.

Animal Safety:

In a margin of safety study, NEXGARD was administered orally to 8- to 9-week-old Beagle puppies at 1, 3, and 5 times the maximum exposure dose (6.3 mg/kg) for three treatments every 28 days, followed by three treatments every 14 days, for a total of six treatments. Dogs in the control group were sham-dosed. There were no clinically-relevant effects related to treatment on physical examination, body weight, food consumption, clinical pathology (hematology, clinical chemistry, or coagulation tests), gross pathology, histopathology or organ weights. Vomiting occurred throughout the study, with a similar incidence in the treated and control groups, including one dog in the 5x group that vomited four hours after treatment.

In a well-controlled field study, NEXGARD was used concomitantly with other medications, such as vaccines, anthelmintics, antibiotics (including topicals), steroids, NSAIDs, anesthetics, and antihistamines. No adverse reactions were observed from the concomitant use of NEXGARD with other medications.

Storage Information:

Store at or below 30°C (86°F) with excursions permitted up to 40°C (104°F).

How Supplied:

NEXGARD is available in four sizes of beef-flavored soft chewables: 11.3, 28.3, 68 or 136 mg afoxolaner. Each chewable size is available in color-coded packages of 1, 3 or 6 beef-flavored chewables.

NADA 141-406, Approved by FDA

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Duluth, GA 30096-4640 USA

Made in Brazil.

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