Anti-Müllerian hormone and inhibin-B concentrations vary cyclically in nonovulating queens within reference ranges established for determining gonadal status in cats

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OBJECTIVE
To define cyclic changes in anti-Müllerian hormone (AMH), inhibin-B, and progesterone concentrations and establish statistically valid, population-based clinical reference ranges in queens.

ANIMALS
Cyclic queens (fertile, n = 6; infertile, 6) from an institutional breeding colony were blood sampled longitudinally, each for over 2 months, between November 2021 and February 2022, and residual serum samples from intact (n = 205) and ovariohysterectomized (49) queens from clinical submissions were used to establish reference ranges for intact and spayed females.

METHODS
AMH and inhibin-B were measured using commercially available ELISAs, progesterone was measured using an in-house ELISA, and 90% CIs were calculated from these data.

RESULTS
AMH and inhibin-B fluctuated in a highly correlated, cyclic pattern in 3 queens that did not ovulate immediately, whereas AMH declined as progesterone increased, indicative of ovulation, which occurred spontaneously early in the sampling period in 3 others; statistically valid reference ranges were established in intact and ovariohysterectomized females.

CLINICAL RELEVANCE
Cyclic changes in hormone profiles were defined, providing relevant context for interpreting results in cases seeking to determine gonadal status (presence or absence of gonadal tissue) on the basis of established, population-based reference ranges reported here for cats for the first time.

Keywords: feline, anti-Müllerian hormone, inhibin-B, reference ranges, gonadal status

Spay-neuter programs have helped reduce the rates of sheltering and euthanasia of unwanted and unowned animals, but ovariohysterectomy (OVH) is not without potential complications, one of which is ovarian remnant syndrome (ORS). Professional guidelines for spay-neuter programs emphasize the importance of verifying the reproductive status (intact vs neutered) in the selection of patients prior to performing laparotomies. This can sometimes pose a diagnostic challenge when evidence of neutering is not obvious (apparent absence of spay scars or absent chips) and where doubt results from an extended interval between a recorded OVH and the reemergence of signs of estrous cyclicity. Endocrine testing, as a diagnostic aid for ORS, has included pituitary protein (luteinizing hormone) and ovarian steroid (estradiol and progesterone) hormones. More recently, assays for anti-Müllerian hormone (AMH), an ovarian protein hormone, have added another promising approach to determining the likely existence of ovarian (and testicular) tissues in cats and dogs as prospective surgical candidates. However, the interpretation of endocrine assays with respect to determining gonadal status (presence or absence of gonadal tissue) is difficult without knowledge of the variability inherent in cyclic females and without well-supported reference ranges established empirically within diagnostic laboratories.
Reproductive function differs substantially between cats and dogs, and that can complicate the clinical interpretation of reproductive hormones in these species. Cats are seasonal and polyestrous until ovulation is induced,\(^9,11\) whereas dogs are nonseasonal and monosexual and ovulate spontaneously.\(^11\) Other subtle, but still potentially important, differences between species may influence how ovarian hormones are secreted. For instance, sex steroids arise primarily from growing follicles in the canine ovary,\(^12\) whereas the ovarian interstitial tissue of cats is capable of significant androgen secretion as is the follicle itself.\(^13\) Hormone secretion by the follicular granulosa cells, as the principal site of synthesis of the protein hormones, inhibins (\(\alpha\) and \(\beta\) isoforms), and AMH, seems conserved among species.\(^14-17\) Nonetheless, species differences may exist in whether AMH secretion is primarily from follicles before (preantral) or after (antral) becoming gonadotropin responsive and in the relative population size of the various follicle classes.\(^15,18\) The feedback regulation of gonadotropins by some ovarian hormones—steroids and inhibins, specifically—may make their secretion necessarily as variable as the gonadotropins themselves. In contrast, AMH concentrations are relatively stable through ovarian cycles in some species,\(^19,20\) perhaps because they are not part of the feedback loop or because secretion is largely from preantral, non–gonadotropin-dependent follicle populations.\(^15\) Still, concentrations of AMH do vary with cycle stage in bitches,\(^22,23\) as with body weight and age.\(^24\) Relatively little is known about how AMH concentrations vary cyclically in individual cats,\(^17,25,26\) or in populations at large, beyond stage of pregnancy\(^17\) and age as factors in domestic queens.\(^25,27,28\) The monitoring of longitudinal (cyclic) changes in concentrations of AMH through estrus in individual queens has not been reported, and none to date have attempted to concurrently measure secretion of other ovarian hormones including progesterone or inhibins.

The interpretation of hormonal information in the context of clinical cases at the most fundamental level begins with valid reference ranges, preferably determined in those laboratories conducting the analysis using whatever is their chosen assay platform, whatever the calibrators.\(^29\) Steroid hormones, being structurally uniform across species, are more readily assayed than are protein hormones like AMH that are species-specific in their peptide sequences (and therefore epitope recognition by antibodies), post-translational modification, and processing.\(^29\) The estimated hormone concentration in absolute terms varies among assay platforms\(^8\) and is especially dependent on the nature of the calibration curve, which, in the case of protein hormones, should ideally comprise a species-homologous reference standard. The use of variable, nonhomologous standard curves, as is the case when utilizing assay platforms designed for human samples, will yield results that are significantly different from studies using assays with alternative calibrators. Concentration estimates of AMH among these platforms on the same samples will not be comparable.\(^30\) Our laboratory recently reestablished reference ranges for canine AMH using a commercially available, canine-specific assay (unpublished observations; author AJC) that exhibited promising evidence of cross-reactivity with feline AMH. The current report provides reference ranges for feline AMH in “canine equivalents.” In addition, just as diagnostic acuity is increased by the clinical history and physical examination findings of a case, endocrine diagnostics examining additional hormonal biomarkers can sometimes add significant clinical value. For instance, concurrent progesterone determinations have been recommended to mitigate false-negative interpretations in evaluating AMH concentrations for diagnosis of ORS in the bitch.\(^3\) Therefore, the current investigation sought to reestablish reference ranges for progesterone concentrations in cats presented for clinical evaluation. Lastly, the concentrations of inhibin-B, an assay recently validated in mares,\(^21\) was explored as another potential biomarker for ORS in which AMH and progesterone results are ambiguous and/or inconclusive.

The present study incorporated longitudinal sampling of nonpregnant queens to assess cyclic variability of AMH, inhibin-B, and progesterone concentrations in individual queens, along with population-based, clinical sampling to establish valid reference ranges. It was hypothesized that hormone concentrations would vary significantly in intact cyclic queens and provide relevant context for interpretation of clinical results on the basis of statistically supported reference ranges. Since the opportunities for establishing reference ranges for cats with confirmed ORS are remote, samples from OVH queens (and a smaller number of castrated toms) were used to determine ranges after gonadectomy. Establishing ranges for neutered and intact females provides context for interpreting results from females that are neither completely lacking ovaries nor still intact with concentrations expected to lie between those ranges.\(^8,32\) The potential value of progesterone and inhibin-B, as additional analytes that might mitigate or better inform false negative or inconclusive AMH interpretations, was also explored.

**Methods**

**Animals**

Twelve cyclic healthy queens aged 2 to 7 years were included in the study. All queens had produced at least 1 litter of kittens in their lifetime prior to inclusion. Queens were maintained in a 14:10-hour light:dark lighting cycle all year. Queens were categorized on the basis of outcomes of recent (within the past 2 years) mating attempts prior to the start of the experiment as fertile (\(n = 6\)) if they achieved pregnancies, or infertile (\(n = 6\)) if multiple matings were unsuccessful. Initial blood sampling for progesterone and behavioral observations confirmed all queens were cyclic at the start of the study (nonluteal). Blood and vaginal cytologies were taken twice weekly between November and February in 2021 or 2022 with IACUC approval (No. 2021-4013). Blood sampling continued in the 6 queens classified as fertile after they underwent OVH toward the end of the experimental interval, though the days of sampling relative to the day of surgery varied. Sometimes OVH was performed on the day of sampling and sometimes in between
Assays
Analyses of serum samples for AMH and inhibin-B were conducted using commercially available ELISA platforms. A canine-specific assay (Canine AMH, AL-116; Ansh Labs) was used to measure AMH. This platform incorporates a double monoclonal sandwich with native canine AMH calibrators and therefore represents "canine-equivalent" concentrations in cat samples. The inhibin-B ELISA (Equine/Canine/Mouse Inhibin B ELISA, AL-163; Ansh Labs) that was used was previously validated for horses in our laboratory. The platform incorporates a double monoclonal sandwich with native canine AMH calibrators and therefore represents "canine-equivalent" concentrations in cat samples. The inhibin-B ELISA (Equine/Canine/Mouse Inhibin B ELISA, AL-163; Ansh Labs) that was used was previously validated for horses in our laboratory.31

Progesterone was analyzed using an ELISA after ether extraction, which was validated previously in a number of species including cats.31 Notably, the cross-reactivity of the antisera in the progesterone ELISA was found to be 0.02% with cortisol.31 Preliminary investigations suggested that a canine-specific AMH assay detected feline AMH and an inhibin-B assay detected feline inhibin-B, with both platforms exhibiting dilution parallelism (data not shown). Dilution parallelism utilized remaining feline sera with high concentrations of AMH and Inhibin-B diluted with sera that had low to undetectable concentrations of each hormone, rather than assay buffer. Assay sensitivity was defined as 2 SDs above the assay blank. The analysis of samples from neutered toms was also included in AMH and progesterone analyses when volumes allowed, in part to verify the contribution of extragonadal sources of each hormone. All samples from the queens that were bled sequentially were run within the same assay. The sensitivity and intra- and interassay coefficients of variation (CVs) for the assays of each hormone were calculated (Table 1).

Table 1—Sensitivity and within assays (intra-CV%) or among assays (inter-CV%) coefficients of variation (CVs) for anti-Müllerian hormone (AMH), inhibin-B, and progesterone.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Sensitivity</th>
<th>Intra-CV%</th>
<th>Inter-CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH</td>
<td>0.05 ng/mL</td>
<td>12.4</td>
<td>19.1</td>
</tr>
<tr>
<td>Inhibin-B</td>
<td>1.0 pg/mL</td>
<td>4.9</td>
<td>12.8</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.25 ng/mL</td>
<td>8.5</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Statistical analysis
Differences between fertile and infertile queens were evaluated by analysis of variance using R statistical programs (aov function; The R Project for Statistical Computing) with fertility status as a fixed factor after testing data for normality and homogeneity of variance (Shapiro, test, Bartlett.test, and LeveneTest functions; The R Project for Statistical Computing). Correlations between AMH and inhibin-B were analyzed within each of the 3 fertile queens that did not ovulate immediately but continued to cycle throughout much of the sampling period using the cor.test function. Hormone concentrations were subjected to log transformation to improve homogeneity of variance and normality of data before analysis, as appropriate. Data from fertile queens that ovulated spontaneously during the sampling period based on elevated progesterone concentrations and with at least 2 samples taken subsequently to confirm the luteal phase were aligned around the initial increase in progesterone (arbitrarily assigned sample day 10), and the effects of time were analyzed as a discrete variable for AMH, inhibin-B, and progesterone in a mixed model with queen as a random variable using the lmer function. Concentrations of AMH, inhibin-B, and progesterone were also evaluated pre- and post-OVH with time as a continuous variable (day of OVH assigned sample day 0), again in a mixed model with queen as a random variable using the lmer function.

Reference ranges for AMH, progesterone, and inhibin-B in intact and spayed queens (age range, 2 to 72 months) were determined using the Reference Value Advisor plugin in Excel (Microsoft Corp). Parametric estimates of 90% CIs were made where supported appropriately by adequate sample numbers, and robust nonparametric intervals were utilized otherwise. The inherent variability in concentrations of AMH, inhibin-B, and progesterone, among sera from queens collected randomly without knowledge of cycle stage, was estimated using samples from the fertile and infertile cyclic females and expressed as an average of the CVs for each individual queen.

Results
The queens classified as fertile were slightly, but not significantly (P = .13), younger than those classified as infertile (3.9 ± 0.5 years vs 5.1 ± 0.6 years, respectively). The fertile queens had higher average concentrations of inhibin-B than the infertile queens (67.7 ± 12.4 pg/mL vs 35.3 ± 6.6 pg/mL, respectively; P = .04, η² = 0.35), but there were no significant differences in concentrations of AMH (P = .18) or progesterone (P = .72). Analysis of the longitudinal sampling data on the fertile queens indicated that 3 of them appeared to experience repeated estrous cycles for several weeks (Figure 1) without exhibiting a luteal phase based on consistently low progesterone concentrations that averaged 0.8 ± 0.05 ng/mL. The concentrations of AMH fluctuated from 2 to 8 ng/mL during this period in a repetitive fashion, comprising 4 to 5 apparent cycles. Behavioral estrus and cornified vaginal cytology were not always closely associated with these cycles (data not shown). However, inhibin-B concentrations exhibited a similar pattern to that seen for AMH, and within these exceptions.

3
3 individuals the 2 hormones were highly \((r = +0.62, +0.68, \text{ and } +0.74)\) and significantly \((P < .001)\) correlated. Cyclicality was interrupted in 2 of these 3 toward the end of the sampling interval when progesterone concentration abruptly increased, indicative of ovulation, just before they underwent OVH. A luteal phase was confirmed by elevated progesterone concentrations in 2 consecutive samples taken before OVH in one of these females, but only 1 sample was obtained before surgery from the other.

The other 3 fertile queens ovulated spontaneously, almost synchronously, within 2 weeks of the start of the sampling interval when progesterone concentration abruptly increased, indicative of ovulation, just before they underwent OVH. A luteal phase was confirmed by elevated progesterone concentrations in 2 consecutive samples taken before OVH in one of these females, but only 1 sample was obtained before surgery from the other.

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P < .001), and the same was true of AMH and inhibin-B for 2 males before and after castration (data not shown).

Reference ranges (90% CIs) for all 3 measured hormones were determined in population-based studies conducted on residual samples from intact and OVH females where volumes permitted (Table 2).

Table 2—Reference ranges based on nonparametric (intact queens) or robust parametric (ovariohysterectomized queens) 90% CIs for AMH (ng/mL), inhibin-B (pg/mL), and progesterone (ng/mL) were assessed using the Reference Value Advisor macro in Excel as described by Geffré et al.32 The number of samples from individual cats (n) used in each analysis are shown below each of the respective ranges. Intact queens ranged from 2 to 72 months in age. The ages of ovariohysterectomized queens were unknown.

<table>
<thead>
<tr>
<th>AMH (ng/mL)</th>
<th>Inhibin-B (pg/mL)</th>
<th>Progesterone (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 205</td>
<td>0.27–18.9</td>
<td>3.72–63.1</td>
</tr>
<tr>
<td>n = 137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovariectomized</td>
<td>0.01–0.16</td>
<td>0.79–13.3</td>
</tr>
<tr>
<td>n = 49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 100</td>
<td></td>
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</tbody>
</table>

Robust reference ranges for AMH and progesterone in serum samples from neutered tomcats were as follows: 0.01 to 0.23 ng/mL (n = 17) and 0.01 to 2.14 ng/mL (61), respectively. The range for AMH in OVH versus intact queens (0.01 to 0.16 ng/mL and 0.27 to 18.8 ng/mL, respectively) did not overlap. The ranges for inhibin-B in OVH and intact females (0.79 to 13.3 pg/mL and 3.27 to 63.1 pg/mL, respectively) overlapped somewhat, and there was extensive overlap in reference ranges for progesterone in OVH and intact queens (0.01 to 7.01 ng/mL and 0.01 to 24.5 ng/mL, respectively). Although AMH declined with age on the basis of linear regression analysis, it was not significant. Nine samples from OVH queens yielded progesterone concentrations from 2 to > 8 ng/mL. These progesterone concentrations were verified by reanalysis, but all had very low AMH concentrations consistent with spayed status, despite the substantial levels of progesterone. The samples from neutered males yielded reference ranges of 0.01 to 0.23 ng/mL for AMH (n = 17) and 0.01 to 2.14 ng/mL (61) for progesterone. There was insufficient serum for inhibin-B analysis in neutered males.

Discussion

The present report provides evidence validating heterologous immunoassays for AMH and inhibin-B in cats based on dilution parallelism, cyclical concentration increases (generally coincident with behavioral and cytological estrus), and the rapid disappearance of both hormones after OVH (Figure 3). Both AMH and inhibin-B appear to provide insights into ovarian follicular activity in cats and offer significant value in the diagnosis of gonadal status based on the reference ranges established here. To the best of the authors’ knowledge, these are the first feline reference ranges for these hormones determined independently and reported. The samples used represented a population of cats, some of unknown health or confirmed reproductive status, possibly posing a limitation of the study. Alternatively, these samples are more likely than not to be representative of those submitted for analysis of gonadal status without accompanying clinical histories (unpublished observations; AUC). There was no overlap between the CIs for AMH in intact versus OVH queens, some overlap of the inhibin-B intervals, and more extensive overlap for progesterone. This was reflected in the CVs calculated for each of these hormones, suggesting that AMH (smallest CV) was the most reliable indicator of the presence of ovarian tissue and inhibin-B assessment is perhaps a useful adjunct to it, depending on the dynamics of secretion during ovulatory and nonovulatory cycles. However, these data suggest that greater caution should be exercised in the interpretation of progesterone. In aggregate, it is concluded that AMH and inhibin-B concentrations can be interpreted with reasonable confidence in determining gonadal status in cats with consideration given to the expected cyclic variations as described here for the first time.

As already noted, the reproductive endocrinology of the queen3,9–10 differs in many respects from that of the bitch,9,11 and therefore assumptions concerning expected cycle stage-related secretory patterns of ovarian hormones, extrapolating from canine to feline, warrant investigation and empirical evidence. It is well-known that, in contrast to spontaneous ovulation in the bitch, ovulation in cats is generally inducible by the physical act of copulation42 or mechanical stimulation of the cervix43,44 as well as the administration of human chorionic gonadotropin or gonadotropin-releasing hormone (GnRH).10,38 Nonetheless, it is also known that a significant proportion of queens can ovulate spontaneously, especially if housed within sight or sound of males,9,20,21 which was confirmed by the results of the current study. Progesterone concentrations > 1 to 2 ng/mL are thought to be a reliable indicator of luteal tissue (depending on the stage of luteal function), ovulation, and thus the presence of functional ovarian tissues in dogs41,42 and cats.10 In this regard, the measurement of progesterone in the bitch has proven to be a valuable addition to the diagnostic investigation of canine ORS,3 mitigating the potential for false-negative results based on AMH determinations alone. The significant rate of spontaneous ovulation in cats suggests that progesterone might prove similarly useful in this species also. Surprisingly, the concentration ranges determined for progesterone in spayed queens (and castrated toms) overlapped considerably with those of intact females, suggesting that their value as an adjunct to AMH in the determination of gonadal status may be limited in cats. Prior studies have demonstrated the ability of the feline adrenal cortex to secrete progesterone in significant amounts under acute stimulation,43 and this may pose a particular problem in sampling strays. Progesterone in a sample taken from cats under stress may not be as reliable as generally believed at the lower concentrations generally considered indicative of luteal tissue.

Diagnosis of canine ORS has been greatly facilitated by the development and utilization of AMH assays4–6 and the establishment of population-based reference

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observed in domestic queens. For AMH, however, this is complicated by the availability and use of different commercial assays, many of which are human oriented, as described and reviewed recently. Different assay platforms exhibit different sensitivities depending on the antisera employed and yield differences in concentration estimates for the same hormone when they employ heterologous or heterogenous calibrators. Whatever the analytical platform used, assay results for AMH are still best interpreted with knowledge of the variability with cyclicity or the likely cycle stage. Among the ovarian hormones available for clinical assessment, AMH is unique in not being a component of the hypothalamic-pituitary-gonadal feedback axis. This may explain why AMH exhibits less variability than other reproductive hormones, including inhibin-B and progesterone, as demonstrated here. This is a particularly important consideration in the diagnosis of gonadal status (suspected ORS, for instance) because it also means that there is little or no functional compensation in the immediate postsurgical period if ovarian tissue is missed when OVH is attempted and it remains viable in situ. To this point, hemovariectomy in mares almost exactly halved AMH concentrations in the days after surgery. Therefore, it is likely that once a new baseline concentration is achieved postsurgically, AMH concentrations will reflect the proportion of ovarian (follicular) tissue remaining in cases of feline (or canine) ORS. Practically speaking then, the smaller the remnant, the lower the concentration of AMH and the more difficult the diagnosis becomes, increasing the chances of false-negative interpretations. The variation in AMH concentrations among individual ORS cases might also be expected to exceed what is seen in intact females because the range in the fraction of viable remnant tissue remaining would likely add to the inherent variability among intact females, which is considerable. This appears to be the case based on CVs of AMH concentrations calculated by the present authors using data from the limited number of cases reported by Gozer et al for intact queens (n = 11) and those with confirmed ORS (9). Despite established reference ranges for intact and OVH queens, the diagnosis of ORS will always be more difficult the smaller the remnant happens to be.

The activity of the hypophyseal-pituitary axis may not be subject to negative feedback from AMH, but the results obtained in these experiments are suggestive of AMH secretion originating from follicles that also secrete inhibin-B and are therefore likely responsive to follicle-stimulating hormone. The high, positive correlation seen between AMH and inhibin-B concentrations during repetitive estrous cycles, without ovulation, provided strong evidence that AMH secretion increases and decreases in concert with follicle growth and regression in queens. These data are consistent with the differences in AMH observed with estrous cycle stage in queens, as previously reported. The expression of α, βA, and βB subunits of inhibin is high in the granulosa layer of antral follicles of cats and inhibin-B secretion increases with antral follicle development in mares. As the current data indicate it does in cyclic queens. Results from other recent studies support this view. A decrease in AMH was observed in domestic queens and female cheetahs after they were treated with a GnRH agonist to down-regulate their reproductive axis. Similarly, there was a decrease in AMH after vaccination of queens against GnRH. Lastly, AMH concentrations (using the same commercial ELISA platform as in the present study) were positively correlated with antral and total follicle numbers in nonpregnant queens. Therefore, systemic AMH concentrations in cats are likely determined primarily by secretion from follicles that are gonadotropin responsive. How this relates to reports that AMH concentrations are higher during anestrous than the follicular phase, but not between interestrus and estrous queens (that were lower than pregnant females), is difficult to reconcile at the present time. Concentrations of AMH peak during canine proestrus and decline in estrus and AMH concentrations declined after ovulation in the queens of the present study, but there was little variation observed with cycle-stage in mares, or women. Clearly there are distinct differences in the developmental stage of follicles responsible for circulating AMH concentrations among species, suggestive of different physiological roles in them that warrant further investigation.

Inhibin-B determined alongside AMH in the cyclic fertile queens provided further context for the endocrine changes accompanying cyclicity. The increase in progesterone concentrations in consecutive samples indicative of a luteal phase following ovulation was useful in normalizing cycles in the fertile queens. Normalizing hormone concentrations around ovulation indicated that AMH was already decreasing in the preovulatory samples as it appears to do in the bitch. In contrast, inhibin exhibits a more complex secretory pattern in the female blue fox when sampled daily. Blue fox vixens experienced a preovulatory peak of inhibin that plummeted rapidly but very transiently after ovulation, before recovering again 1 or 2 days later. Sampling twice a week would not suffice to define such dynamic fluctuations in concentrations of inhibin-B if the same were true of cats and may explain the lack of definitive changes in concentrations around the time of ovulation in this study. As with progesterone, the adrenal gland may also have the capacity for inhibin secretion, as has been demonstrated in Cushing’s disease in dogs. Given the rarity of Cushing’s disease in cats, however, elevated concentrations of inhibin-B that exceed the reference range determined here for OVH queens may be useful evidence of the existence of ovarian tissue for cases in which AMH is inconclusive.

As already noted, there is significant value in determining the gonadal status of unowned or stray queens surrendered to shelters (and others encountered in practice). The establishment of reference ranges in intact and gonadectomized cats using canine-equivalent AMH, progesterone, and inhibin-B determinations provides clarity to how reliably these biomarkers can be used in clinical diagnosis. Less variability in AMH than either inhibin-B or progesterone suggests it is the more reliable, diagnostic indicator of gonadal status in queens. The data presented here also remind us that no single test is perfect or provides unequivocal results in all cases. Therefore, although the
determination of gonadal status is aided by endocrine testing, it clearly benefits when results are consistent with a reproductive history (if, or where, known) and reproductive examination findings including behavior and vaginal cytology at a minimum. Repeat testing is advised when endocrine results and clinical findings are equivocal or in conflict. Whether to perform an exploratory laparotomy is not a trivial question. With respect to ORS, the smaller the remnant, the harder it is to diagnose, visualize by imaging, and find at surgery, especially without the presence of the uterine horns for guidance and since it may be located anywhere in the peritoneal cavity. Moreover, surgical times are extended when ovarian tissues cannot be found. Therefore, the decision to explore surgically is best made with a maximum of complementary, and ideally consistent, clinical and endocrine information. The disappearance of AMH from a serum sample taken 7 to 10 days postsurgically may also be useful in confirming the success of the removal of remnant tissue that otherwise would necessitate histopathology.

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