Vaginal impedometry for detection of optimal breeding time in bitches

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Objective—To compare the efficacy of canine vaginal impedometry in identifying the preovulatory luteinizing hormone (LH) peak to that of currently used methods (serum progesterone concentration measurement, vaginal cytologic evaluation, and vaginoscopy).

Design—Prospective study.

Animals—12 sexually intact female dogs.

Procedures—12 mature postpubertal Beagle (n = 3), Beagle-cross (2), and hound-cross (7) bitches ranging from 7.5 to 275 kg (16.5 to 60.6 lb) were enrolled in the study. After the onset of spontaneous proestrus, determined on the basis of appearance of serosanguineous vaginal discharge, serum progesterone assays, vaginoscopy, vaginal cytologic evaluation, and vaginal impedometry were performed daily until approximately 4 days after peak LH concentration (day 0) as measured by radioimmunoassay. Vaginal impedometry was compared against serum progesterone concentration measurement, vaginal cytologic evaluation, and vaginoscopy as a method for accurately identifying the LH peak and therefore the optimal breeding time. Ten of 12 bitches were bred with subsequent assessment of embryos.

Results—Vaginal impedometry accurately predicted the preovulatory LH peak in 5 of 11 bitches. One bitch was removed from the study because data were not collected. Of the remaining 11 bitches, 6 had their LH peak on the day serum progesterone concentration first exceeded 2 ng/mL. Crenulation scores reached 1 (mean, 1.3; 95% confidence interval, 0.8 to 1.7) on day 0 as expected; however, these scores were not significantly different from those on days –1 or 1. Vaginal epithelial cell populations did not change noticeably on day 0. Nine of the 10 bitches that were bred produced viable embryos.

Conclusions and Clinical Relevance—Results suggested that daily use of vaginal impedometry in bitches was unreliable as a method for monitoring periovulatory events. All techniques evaluated (ie vaginal impedometry, serum progesterone concentration assays, vaginoscopy and vaginal cytologic evaluation) frequently produced inaccurate results when used individually. Multiple methods should be used to identify optimal breeding time in dogs. (J Am Vet Med Assoc 2014;245:1360–1366)

Vaginal mucus impedance, the electrical resistance of vaginal mucus, may be closely correlated with serum concentrations of progesterone and estradiol.1–3 Opinions differ as to whether monitoring VMI by means of vaginal impedometry could provide an accurate and specific indication of the optimal time to breed in bitches1 or whether this method is only useful for identification of the stage of the estrous cycle of a bitch.2 In sheep,3 cattle,4 pigs,5 and monkeys,6 vaginal impedometry has been used to successfully determine the time of ovulation, as indicated by low electrical resistance during optimal breeding periods. However, bitches have been found to have high electrical resistance during optimal breeding periods, a difference which may be attributed to their unique serosanguineous discharge.1,2 Additionally, bitches range widely in size and a single impedometer is unlikely to reach a consistent location among all breeds, which may make measurement of VMI inconsistent and vaginal impedometry unreliable as a method for daily monitoring of periovulatory events in this species.3,7

Currently used methods of monitoring periovulatory events to identify the optimal time to breed in dogs include serum or plasma progesterone assays, vaginal cytologic evaluation, and vaginoscopy. A serum progesterone concentration > 2 ng/mL is typically accepted as an indicator of the LH surge,8–11 yet a wide range (3

ABBREVIATIONS

CI  Confidence interval
CLIA  Chemiluminescence immunoassay
LH  Luteinizing hormone
VMI  Vaginal mucus impedance

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to 10 ng/mL) of progesterone concentrations has been reported as indicative of ovulation. These concentrations can be variable among bitches, laboratories, type of assay used, and methods of specimen handling, and may depend on whether progesterone is measured in plasma or serum. Changes in both vaginal mucosa and epithelium are influenced primarily by circulating estradiol concentrations, which can be variable within and among bitches. In sheep, progesterone concentrations primarily influence VMI, except when progesterone concentrations are low, at which point the estradiol-to-progesterone concentration ratio also influences VMI. If this is also true in bitches, vaginal impedometry could provide a more consistent method for monitoring peri-ovulatry events than methods influenced primarily by estradiol, such as vaginal cytologic evaluation and vaginoscopy.

The optimal time to breed is typically 4 to 7 days after the LH surge, which requires precise monitoring. Both of the vaginal impedometry studies previously performed in bitches have relied on surrogate indicators of the LH surge or time of ovulation (eg, serum progesterone assays) to evaluate the efficacy of this technique for monitoring peri-ovulatory events. Critical assessment of these monitoring methods by comparison with direct measurements of LH concentration may enable improvements in breeding management and use of assisted reproductive technologies. Therefore, the objective of the study reported here was to compare the efficacy of canine vaginal impedometry in identifying the preovulatory LH peak against that of currently used methods, including serum progesterone concentration measurement, vaginal cytologic evaluation, and vaginoscopy. We hypothesized that use of vaginal impedometry in conjunction with these other methods would be more accurate than vaginal impedometry alone.

**Materials and Methods**

**Experimental animals**—All experimental procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the Institutional Animal Care and Use Committee at the University of Guelph. Twelve mature postpubertal bitches were enrolled in the study. Bitches were Beagles (n = 3), Beagle crosses (2), or hound crosses (7) with a median weight of 21 kg (46.3 lb; range, 7.5 to 27.5 kg [16.5 to 60.6 lb]). After the onset of spontaneous proestrus, identified on the basis of appearance of serosanguineous vaginal discharge, all animals were housed at the Central Animal Facility for the duration of the study.

**Vaginal impedometry**—Vaginal impedometry was performed with a 2-electrode, rigid, battery-operated impedometer. Vaginal mucus impedance was measured and recorded daily. The vaginal impedometer was completely inserted into the vagina and spun in one direction to ensure that the electrodes came into full contact with fresh mucus. The impedometer device displays a value that is a mean calculated on the basis of several readings. Those raw values are not available to the operator. Thus the displayed mean VMI was recorded. This process was repeated, ensuring the impedometer was first turned to expose the electrodes to new mucus. After each daily use and also in between bitches being examined on the same day, the impedometer was disinfected with 70% isopropyl alcohol, followed by concentrated algaecide and then rinsed with distilled water. Vaginal impedometry data for 1 of the 12 bitches were not included because, after the data from this animal were obtained, it was determined that VMI had not been measured on the correct dates corresponding to the LH peak. As such, all VMI data for this bitch were excluded from the analyses.

Daily VMI readings were used for statistical analyses. In addition, VMI readings for each bitch were plotted in a spreadsheet software program to allow observation of graphical trends among days from the LH peak. Peak VMI was defined as the highest VMI among days examined. Peak resistance was expected to occur 2 or 3 days after the LH peak.

**Hormonal assays**—As previously described, blood samples were collected once daily for the detection of the onset of proestrus until approximately 4 days after the LH peak. Serum was assayed by means of a CLIA for progesterone concentration on the day of collection (for the first day of examination and then every second day) at the Animal Health Laboratory. The remaining serum was stored at –20°C until used for radioimmunoassay. Daily serum progesterone and LH concentrations were later determined by means of a radioimmunoassay validated for use in canine species at the Animal Health Diagnostic Center. Progesterone concentrations assays were retrospectively found to have been measured on the day of the LH peak for 6 of 12 bitches.

The LH peak (day 0; as measured by radioimmunoassay) was used as a reference, as previously described, to determine whether vaginal impedometry, progesterone assays, vaginoscopy, and vaginal cytologic evaluation were accurate in identifying the optimal time to breed in bitches.

**Vaginoscopy**—Vaginoscopy was performed daily with a pediatric proctoscope. The proctoscope was stored in didecyl dimethyl ammonium chloride and N-alkyl dimethyl benzyl ammonium chloride. Before use, the proctoscope was rinsed with tap water followed by irrigation with sterile saline (0.9% NaCl) solution. Vaginal crenulation was assessed in the cranial aspect of the vagina with the dorsal caudal median fold in view. Descriptions of the changing appearance of vaginal mucosa throughout the estrous cycle were assigned a crenulation score on a scale from 0 to 3: 0 = pale red edematous mucosa (expected prior to the pre-ovulatory LH peak), 1 = shrinking pale mucosa without angulation (expected at the preovulatory LH peak), 2 = shrinking pale mucosa with further angulation (expected at ovulation), or 3 = maximal wrinkling and pale mucosa (expected at oocyte maturation and maximum fertility). After observing crenulation, cotton-tipped swabs moistened with tap water were inserted through
the proctoscope and rotated in one direction to obtain cells for vaginal cytologic evaluation.

**Vaginal cytologic evaluation**—Vaginal cytologic slides were collected and evaluated as previously described. Slides were stained with a modified Wright-Giesma stain. A slide characteristic of estrus had 100% superficial cells, >80% pyknotic or absent nuclei, no polymorphonuclear cells, and little or no background debris.

**Artificial insemination**—As a component of a separate research experiment (unpublished data), 10 of the 12 bitches were artificially bred with fresh semen. Optimal breeding time was identified on the basis of results of progesterone assays, vaginoscopy, and vaginal cytologic evaluation. Eleven to 15 days after the LH peak (as measured by radioimmunoassay), ovariohysterectomy was performed. For the 10 bitches that were bred, the uterine horns were flushed for embryos and the collected embryos were visually assessed.

**Statistical analysis**—Vaginal impedometry analyses were performed in 2 ways. A general linear mixed model was fitted. The 2 daily VMIs for each bitch were input into the statistical software, and mean daily VMIs were used for analysis. The fixed effect was the day of the LH peak. The model was simplified by removing nonsignificant terms. Because repeated measurements were performed in the animals, a potential autocorrelation needed to be accounted for. Correlation error structures were attempted, as described for vaginal impedometry. Residual analyses, also as described for vaginal impedometry, were conducted to examine the ANOVA assumptions. Least square means were calculated for each day from the preovulatory LH peak and the differences (for untransformed data) or ratios (for transformed data) of least square means were used to determine significant (P < 0.05) differences between days, by means of the Tukey-Kramer method. For vaginal cytologic evaluation, median and 95% CI were calculated by means of the median unbiased estimate method.

**Results**

**Vaginal impedometry**—For the general linear mixed model analysis, VMIs were log transformed, resulting in all assumptions of the model being met. The random error structure produced the lowest Akaike information criterion (Table 1). Briefly, daily median VMI ranged from 281 (day –2) to 562 (day 2). Significant increases in median VMI measurements were observed among several days. The highest median VMIs were reached on day 2; however, median VMI on this day was not significantly different from median VMIs on previous or subsequent days.

From the analysis with exact binomial CIs (Sterne method), our data suggested that vaginal impedometry was an accurate method of monitoring periovulatory events in only 5 of 11 bitches, or 45% of animals (with Sterne limits of 20% and 74%; Figure 1). Bitches that did not have VMI peak at the correct time did not have a peak at all, or had a large false peak (Figure 2).

Table 1—Daily VMI readings in Beagle (n = 3), Beagle-cross (2), and hound-cross (7) bitches with weight ranging from 16.5 to 60.6 kg (7.5 to 27.5 kg).

<table>
<thead>
<tr>
<th>Day</th>
<th>Median VMI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>–3</td>
<td>341a,b</td>
<td>230 to 505</td>
</tr>
<tr>
<td>–2</td>
<td>349a</td>
<td>193 to 412</td>
</tr>
<tr>
<td>–1</td>
<td>405a,b</td>
<td>275 to 506</td>
</tr>
<tr>
<td>0</td>
<td>536b</td>
<td>364 to 790</td>
</tr>
<tr>
<td>1</td>
<td>449a,b</td>
<td>305 to 661</td>
</tr>
<tr>
<td>2</td>
<td>562b</td>
<td>382 to 837</td>
</tr>
<tr>
<td>3</td>
<td>491a,b</td>
<td>334 to 723</td>
</tr>
<tr>
<td>4</td>
<td>341a</td>
<td>230 to 505</td>
</tr>
</tbody>
</table>

Day 0 represents the preovulatory LH peak. All values reported have been back transformed from the log scale.

*Values with a common superscript letter differ significantly (P < 0.05)
Hormonal assays—Each bitch was found to have anticipated patterns of hormone secretions, as previously reported. Mean ± SD progesterone concentration (as measured by means of radioimmunoassay) on day 0 was 3.07 ± 1.06 ng/mL. On day 2, the day of presumed ovulation, mean ± SD progesterone concentration was 6.01 ± 2.11 ng/mL, as previously reported. Mean ± SD CLIA progesterone concentration was 3.90 ± 1.56 ng/mL on day 0 and 5.60 ± 2.28 ng/mL on day 2. Only 6 of 11 bitches had their LH peak (day 0) occur on the day radioimmunoassay progesterone concentrations first exceeded 2 ng/mL. This analysis could not be performed for CLIA progesterone concentrations because these were not performed daily. On the basis of an LH concentration cutoff of 1 ng/mL to identify the LH surge and the highest assayed LH concentration to identify the day of the LH peak, 10 of 12 bitches had their LH peak and surge on the same day and 2 of 12 bitches surged the day before their peak. Taking doubling of radioimmunoassay serum progesterone from one day to the next as an indication of LH surge, only 6 of 12 bitches would have had their LH surge properly identified. Progesterone concentration doubled in 2 bitches the day after the LH peak (or surge); in 2 bitches, progesterone concentration never doubled from one day to the next during the course of the study (up to 4 days past the LH peak).

Vaginoscopy—for the general linear mixed model analysis, all assumptions of the model were met and transformation of the data was not necessary. The random error structure produced the lowest Akaike information criterion (Table 2). Briefly, daily mean crenulation scores ranged from 0.1 (95% CI, 0.1 to 0.6) on day –3 to 3.0 (95% CI, 2.5 to 3.5) on day 4. There were significant increases in mean crenulation scores observed among days, which progressively increased from day –3 to 4. Crenulation scores reached 1 (mean, 1.3; 95% CI, 0.8 to 1.7) on the day of the LH peak, as expected; however, scores on day 0 were not significantly different from scores on either day –1 or 1.

Vaginal cytologic evaluation—All 12 bitches had vaginal cytologic findings characteristic of estrus before the day of their LH peak. The vaginal epithelial cell population viewed on the cytologic slides did not change noticeably at the LH peak.

Artificial insemination—Of the 10 bitches bred, 9 produced viable embryos, confirming the accurate detection of optimal breeding time, on the basis of directly measured LH concentration as a reference.
Discussion

The results of the present study of 12 mature postpubertal bitches suggested that daily vaginal impedometry is unreliable as a method for monitoring of periovulatory events. All techniques evaluated (ie, progesterone assays, vaginoscopy, vaginal cytologic evaluation, and vaginal impedometry) frequently produced inaccurate results when used individually. The impedometer user manual6 states that the profile trace of the VMI readings is important, not the numerical values. The manual also warns users of a false peak, which may occur before and at a lower resistance than the real peak. In this study, a peak was thereby defined as the highest VMI reading retrieved among days examined. Peak resistance occurs 2 or 3 days after the LH peak and the optimal time to breed is 2 days after the peak VMI reading.24 Thus, if bitches had VMI peaks occurring on days 2 or 3 after the LH peak, this technique was categorized as efficacious for monitoring periovulatory events and identifying the optimal time to breed in bitches. Although the highest median VMI was reached on day 2, median VMI on this day was not significantly different from values obtained on previous or subsequent days.

Additionally, in the present study, only 5 of 11 bitches had their peak VMI measurement occur at the appropriate time according to the impedometer user manual24 (Figure 1). Bitches that did not have VMI peak at the correct time had the peak occur too early, did not have a peak at all, or had a large false peak (Figure 2). This false peak would complicate the use of vaginal impedometry for monitoring periovulatory events because even if there were an increase in VMI at the appropriate time (day 2 or 3), if this increase were not larger than the false peak, the user would incorrectly (prematurely) identify the optimal time to breed. Additionally, if this method were being used to determine the optimal time to breed artificially (eg, with fresh semen), there would be little time to prepare for breeding because the user would need to first wait to observe whether VMI reached a peak (ie, wait until VMI declines).

Furthermore, the ability of the impedometer to measure the resistance of pericervical mucus may be compromised by the length of the device, which is too short to reach the cranial vagina in any bitch larger than a Beagle. However, our data did not suggest a compromised ability to determine the optimal time to breed because the 3 bitches that had their peak VMI occur at the appropriate time were of varying size and weight (7.5 to 27.5 kg [16.5 to 60.6 lb]).

Although the impedometer user manual24 states that the unit of measurement of VMI is unimportant to the analyses, and that VMI is simply reported in arbitrary units, it should be noted that a prior study describes trends in VMI in Ohms. Vaginal mucus impedance measurements as previously reported have been shown to reach a plateau lasting 4 to 11 days.2 Because the optimal time to breed has been identified during peak VMI and these peak values reach a plateau, it has been suggested that recording values in 2-day intervals is sufficient to determine the optimal time to breed.1 This plateau in VMI was not observed in the present study, suggesting that daily examinations would be required.

In sheep, VMI is primarily influenced by progesterone concentrations but is also affected by the ratio of estradiol to progesterone concentration when progesterone concentrations are decreasing or are low.3 Vaginal mucus impedance may be controlled by estradiol concentration in bitches, an assumption that has been made based on the parallel changes observed for results of vaginal cytologic evaluation and for vaginal impedance, which are primarily affected by estradiol concentration.3 Because decreased estradiol concentration have been reported to occur near the LH peak,12,13 the results of our study, which found no significant relationship between LH and VMI, suggest that vaginal impedance was not controlled by estradiol concentration. It may be that vaginal impedance was controlled primarily by progesterone concentration or, as it is in sheep,7 by the progesterone-to-estradiol concentration ratio. The unique pattern of changing progesterone concentrations in bitches (a result of preovulatory luteinization of follicles) could be responsible for the unique pattern of changes in VMI, compared with other species; however, this would require further investigation.

Although both CLIA and radioimmunoassay progesterone concentrations for all bitches exceeded 2 ng/mL on the day of the LH peak, only 6 of 11 bitches had their LH peak occur on the day radioimmunoassay progesterone concentrations first exceeded 2 ng/mL. It should be noted that although the changes in hormonal concentrations observed among days from the LH peak were as anticipated (on the basis of a previous report29), the values on the days of interest (day 0 and 2) did not allow for precise identification of the LH peak and ovulation. This demonstrated that measurement of progesterone concentration could serve as an estimate of the LH peak but should not be used to reliably identify the optimal breeding time when used alone. On the basis of the variability in progesterone concentrations between CLIA and radioimmunoassay, the day that progesterone concentrations first exceeded 2 ng/mL may differ among assays, making identification of the LH peak inconsistent.

Measurement of progesterone concentration is often used as an indirect indicator of the LH surge because it is more economical to assay.30 Our data support previous speculation that sole use of progesterone concentration cutoffs to predict the LH surge may be unreliable,30 in that we could not reliably use traditional methods of cutoffs or doubling of progesterone concentration to correctly identify the LH surge. This could be for a number of reasons, many of which have been previously documented, including variability among bitches, laboratories, the type of assay used, specimen handling, and whether plasma or serum is used.8,12-18 Progesterone assays should certainly be used in ovulation monitoring but in tandem with other measures, perhaps including serial ovarian ultrasonography25 or in-house LH assays.

A progressive increase in crenulation scores relative to days from the preovulatory LH peak was observed in this study, which made it difficult to know when specific periovulatory events had occurred. The crenulation score did reach 1 (mean, 1.3; 95% CI, 0.8 to 1.7) on the day of the LH peak, as expected. However, the crenulation score on day 0 was not significantly different from
the crenulation scores on either day –1 or 1, making the precise detection of the LH peak unlikely. Crenulation is influenced by declining estradiol concentrations and is subjective in interpretation of the degree of wrinkling. These characteristics make evaluation of crenulation a variable and unreliable method of monitoring periovulatory events when performed alone, as has been previously reported.

Vaginal cytologic evaluation was ideal for determining what stage of the estrous cycle a bitch was in, specifically when a bitch was entering into estrus. However, there was no noticeable change in the population of vaginal epithelial cells at the LH peak or at presumed ovulation, making this technique unsuitable for accurately identifying the optimal time to breed in bitches if used alone.

In this study, we experienced the same difficulties as have been previously documented with vaginal cytologic evaluation. For example, variability in the amount and time maximum cornification is visible in a vaginal smear varies among bitches, making it difficult to know the exact time for optimal breeding. Error has also been documented when the appearance of anucleate cells is relied on as an indication of the progression from proestrus to estrus. In addition, the type of stain used can introduce variability in determining whether a cell appears as anucleate or not. Vaginal cytologic evaluation is ultimately a retrospective method of monitoring periovulatory events in bitches. Monitoring the progression from estrus to diestrus (when superficial cells begin to decrease) can allow for retrospective confirmation of whether breeding was performed at the optimal time. This method has been shown to be reliable (if performed frequently) to determine the stage of the estrous cycle but not to precisely or prospectively determine the optimal time to breed.

It should also be noted that measurements of VMI and crenulation scores were both acquired easily. However, measurement of progesterone concentrations first required serum to be separated from blood samples and then assayed. Use of the impedometer was also less tedious than creating and evaluating a vaginal cytologic slide, which used disposable swabs to retrieve cells, which were then transferred onto a slide and stained, followed by microscopic evaluation. Both the impedometer (used for vaginal impedometry) and the pediatrix proctoscope (used for both vaginal cytologic evaluation and vaginoscopy) required thorough disinfection after each use. Disinfection is a concern when these pieces of equipment are used because they have the potential to spread infectious agents. Vaginal impedometry and progesterone assays produce raw values as assessed by a device, whereas vaginal cytologic evaluation and vaginoscopy both require visualization and interpretation by someone knowledgeable and experienced. This subjective interpretation may be inconsistent among users and among days with the same user.

All techniques required serial monitoring for optimal reliability. Thus, it is recommended that the accuracy of identifying optimal time to breed, level of invasiveness, and ease of use of each technique, in addition to available resources, be considered when determining monitoring methods. Vaginal impedometry, progesterone assays, vaginal cytologic evaluation, and vaginoscopy all have advantages and disadvantages for use in determining the optimal time to breed in bitches. The results of the present study suggested that vaginal impedometry would not be accurate when used alone to identify optimal breeding time and did not improve accuracy even when used in combination with the other currently used methods. By critically assessing these monitoring methods on the basis of quantitative measurement of LH concentration as a reference, they can be accurately reviewed, which may allow for improvements in breeding strategies and improved success of assisted reproductive technologies in dogs.

References

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From this month’s AJVR

**Effectiveness of a steam cleaning unit for disinfection in a veterinary hospital**

Cheryl L. Wood et al

**Objective**—To evaluate whether the application of steam to a variety of surface types in a veterinary hospital would effectively reduce the number of bacteria.

**Sample**—5 surface types.

**Procedures**—Steam was applied as a surface treatment for disinfection to 18 test sites of 5 surface types in a veterinary hospital. A pretreatment sample was obtained by collection of a swab specimen from the left side of each defined test surface. Steam disinfection was performed on the right side of each test surface, and a posttreatment sample was then collected in the same manner from the treated (right) side of each test surface. Total bacteria for pretreatment and posttreatment samples were quantified by heterotrophic plate counts and for *Staphylococcus aureus, Pseudomonas* spp, and total coliforms by counts on selective media.

**Results**—Significant reductions were observed in heterotrophic plate counts after steam application to dog runs and dog kennel floors. A significant reduction in counts of *Pseudomonas* spp was observed after steam application to tub sinks. Bacterial counts were reduced, but not significantly, on most other test surfaces that had adequate pretreatment counts for quantification.

**Conclusions and Clinical Relevance**—Development of health-care–associated infections is of increasing concern in human and veterinary medicine. The application of steam significantly reduced bacterial numbers on a variety of surfaces within a veterinary facility. Steam disinfection may prove to be an alternative or adjunct to chemical disinfection within veterinary practices. (*Am J Vet Res* 2014;75:1083–1088)