Assessment of Vcheck® analyzer for rapid progesterone concentration measurement including recommendations for achieving the optimal breeding time in bitches

Supphathat Wutthiwitthayaphon1, Thanikran Suwannachote2, Saengtawan Arayatham3,4, Wisut Prasitsuwan3, and Sakchai Ruenphet1,3

Materials and Methods: Ninety-four serum samples from bitches were analyzed using the Vcheck® analyzer and compared with CMIA. Thorough documentation included the mean, standard deviation, 95% confidence interval (CI), and minimum and maximum values of serum progesterone concentrations. Furthermore, Pearson’s correlation coefficient, Lin’s concordance correlation coefficient, and the bias correction factor were meticulously recorded.

Results: The mean progesterone concentration measured using the Vcheck® analyzer was significantly lower than that measured using CMIA, with a mean difference of 1.26 ng/mL of serum. The Bias correction factor was 0.935, which was nearly 1.00, indicating that the line of best-fit was on the perfect line of agreement, providing insight into the measurement accuracy. Pearson’s correlation coefficient, a measure of precision, was also close to 1 (0.939), confirming the reliability of the data. Furthermore, Lin’s concordance correlation coefficient was 0.877, indicating a fair overall agreement between the Vcheck® and CMIA methods. These results support the validity of the Vcheck® analyzer’s results. The present study was developed by aligning with established CMIA guidelines and adapting them using the range and 95% CI derived from each set of results, ensuring a standardized and rigorous approach.

Conclusion: The Vcheck® analyzer provides a rapid assessment of serum progesterone concentration in bitches, with results comparable to those measured using the CMIA technique. However, when considering the use of the Vcheck® analyzer, it is recommended that the results should be interpreted carefully and the interpretation guidelines should be followed. In conclusion, Vcheck® provides a reliable and convenient method for veterinarian practitioners to measure canine progesterone levels in a clinical/hospital setting.

Keywords: chemiluminescent microparticle immunoassay, optimal breeding time, progesterone, Vcheck®.

Introduction

The optimal breeding time in bitches requires an assessment of serum progesterone concentrations [1]. However, this evaluation serves multiple essential purposes, including identifying reproductive irregularities, such as hypoluteoidism [2], and confirming luteolysis before parturition [3–5]. Notably, a characteristic increase in serum progesterone concentrations during the estrus period often exceeding 1 ng/mL, indicates significant hormonal changes. In bitches, ovulation typically occurs 36–50 h after the luteinizing hormone (LH) peak [6], which correlates with serum progesterone concentrations of approximately 2.02 0.18 ng/mL [7]. These concentrations then escalate to a range of 4.00–10.00 ng/mL on the day of ovulation [8], indicating a significant hormonal shift and the onset of ovulation. Intriguingly, despite this range, Seefeldt et al. [9], Marseloo et al. [10], and
Mir et al. [11] suggested a serum progesterone concentration of 5.00–8.00 ng/mL, introducing contrasting perspectives on determining the ovulation.

Various techniques, such as radioimmunoassay (RIA) [12, 13], liquid chromatography-tandem mass spectrometry (LC-MS) [14, 15], and chemiluminescence immunoassay (CLIA) [14, 16], are used to measure serum progesterone concentration in veterinary medicine. An accurate and reliable alternative is the CLIA method, adept at assaying serial blood samples with guaranteed safety, speed, accuracy, and repeatability [17]. CLIA addresses the drawbacks associated with RIA and enzyme immunoassay and serves as a robust solution for precise serum progesterone monitoring, aiding in accurate ovulation prediction and confirmation. Moreover, recent advances, such as point-of-care analyzers, have transformed the measurement of progesterone. These advancements, including rapid fluorescence immunochromatography assay, surface plasmon field-enhanced fluorescence spectroscopy, lateral flow immunochromatography, and competitive enzyme-linked fluorescence assay [18–21], enhance serum progesterone monitoring, ultimately improving veterinary practice in managing reproductive processes.

However, differences in serum progesterone levels are due to the use of different laboratory techniques and variations between bitches. As a result, the precise identification of the ideal mating period necessitates the collection of multiple consecutive blood samples during both the proestrus and estrus phases, which can then be compared with established gold standards or reference laboratory procedures. This investigation involves a comparative analysis of serum progesterone findings derived from a commercial point-of-care analyzer, namely Vcheck®, in contrast to those acquired through chemiluminescent microparticle immunoassay (CMIA) using the same serum samples.

**Materials and Methods**

**Ethical approval and informed consent**

The study was approved by Animal Research Ethics Committee of the Faculty of Veterinary Medicine at Mahanakorn University of Technology, Thailand (approval number ACUC-MUT-2020/006). The owners of the bitches expressed consent to participate in the research by signing an official document.

**Study period and location**

Blood samples were obtained for analytical purposes from August 2020 to July 2023. This acquisition took place at two locations: the Small Animal Teaching Hospital at the Faculty of Veterinary Medicine, Mahanakorn University of Technology, Thailand, and Vet Home Polyclinic in Bangkok, Thailand.

**Sample collection and progesterone measurement**

Ninety-four serum samples were collected from bitches of various ages and breeds, including American bullies, English bulldogs, French bulldogs, Shetland sheepdogs, miniature American shepherds, Cavalier King Charles spaniels, Chihuahuas, Pomeranians, Chow Chows, Akitas, and Pugs. To ensure a comprehensive analysis, all bitches underwent both progesterone concentration determination and vaginal cytology examination, aligning with the study’s multifaceted approach. The aim of this dual approach was to provide a comprehensive understanding of hormonal changes and reproductive stages. The criteria for sampling were defined by vaginal cytology; specifically, 70% cornified epithelial cells were observed on the 1st day, indicating late proestrus. Consequently, a blood sample was collected for progesterone determination to capture hormonal changes during this transitional phase. Subsequently, additional samples were collected on the day when vaginal cytology showed 90% cornified epithelial cells, signifying the onset of the estrus period. This transition from late proestrus to estrus allowed a comprehensive assessment of progesterone fluctuations in different reproductive cycle stages. Two aliquots of serum were meticulously prepared for each bitch sample. One aliquot was immediately used to assess progesterone concentration using CMIA, ensuring immediate measurements for real-time analysis. An Architect i2000SR Immunoassay Analyzer (Abbott Laboratories, Illinois, USA) along with the Architect Progesterone Reagent (Abbott Laboratories, Illinois, USA) was used for this process. Simultaneously, the second aliquot was judiciously stored at 20°C until it was required for assessment. Strategic storage was implemented to facilitate subsequent evaluations performed using commercial point-of-care analyzers, specifically the Bionote V200 analyzer (Bionote, Minnesota, USA) with Vcheck® canine progesterone (Bionote, Minnesota, USA). This allowed a comparative analysis using different assessment methods for a more accurate investigation. The study maintained strict adherence to the manufacturer’s recommendations for laboratory and point-of-care assessment. This commitment has ensured the reliability, accuracy, and validity of the obtained progesterone data and provided a basis for credible findings and conclusions.

**Statistical analysis**

In this study, we conducted a comprehensive analysis of serum progesterone concentration from the collected samples, encompassing the calculation of key statistical parameters, including the mean, standard deviation (SD), 95% confidence interval (CI), minimum (Min), and maximum (Max) value. Rigorous quantification was meticulously conducted across the significant phases of the bitch’s reproductive cycle, including proestrus, LH peak, pre-ovulation, ovulation, post-ovulation, and all stages. A paired t-test was used to assess the presence of a significant difference between CMIA and Vcheck® means. All analyses were rigorously conducted utilizing the free trial version of XLSTAT in Microsoft Excel Home.
and Student Edition (WA, USA) (https://www.xlstat.com/en/download). The designated significance level was set at p < 0.05.

We conducted a comparative analysis with CMIA, a widely recognized reference method, to comprehensively evaluate the performance of Vcheck®. We employed Pearson’s correlation coefficient, a robust statistical tool, to assess the precision of both methods. To provide a more comprehensive view, we utilized Lin’s concordance correlation coefficient, which is known for its ability to rigorously evaluate agreement, to determine the agreement level between Vcheck® and CMIA. It should be noted that perfect concordance is represented by a value of 1, indicating a perfect match between the two methods. In our evaluation, we also considered the bias correction factor as a critical measure of accuracy. Passing-Bablok regression and Bland-Altman analyses were performed to enhance further our understanding and comparison of Vcheck® and CMIA values. These methods provide valuable insights into any systematic differences between the two techniques. These visuals vividly illustrate the outcomes of our analyses, making complex data more accessible and informative to readers.

Results

Table-1 provides a comprehensive overview of the means, SD, 95% CI, and range of serum progesterone concentrations. These values were determined through measurements taken during various phases, including early proestrus, LH peak, pre-ovulation, ovulation, post-ovulation, and all bitch periods. This detailed presentation enables us to understand the distribution of progesterone levels in different phases.

We found an intriguing finding when we compared these values: The average values of all samples obtained using Vcheck® were significantly lower than those obtained using CMIA, with an average difference of 1.26 ng/mL (Table-2 and Figure-1). This difference is not only statistically significant but also significantly impacts the accuracy and precision of the measurements.

The bias correction factor, an essential component in our evaluation, was measured at 0.935, which was nearly 1.00. This proximity to 1.00 indicates that the best-fit line corresponds closely to the perfect line of agreement. This finding strengthens our confidence in measurement accuracy (Table-2 and Figure-2).

In addition, Pearson’s correlation coefficient reached a value of 0.939, confirming the very good precision of our measurements (Table-2). This high level of precision enhances the credibility of our findings.

In addition, Lin’s concordance correlation coefficient was found to be 0.877, indicating fair overall agreement between the Vcheck® and CMIA techniques (Table-2 and Figure-2). This metric adds another layer of validation to our assessment, demonstrating a fair level of agreement between the two techniques.

Discussion

An accurate and impartial assessment of progesterone concentrations is essential when assessing the reproductive status of bitches, in particular when determining the optimal breeding time and when forecasting or supervising the parturition date. RIA methods have been considered the gold standard for quantifying levels in bitches over the years [7, 22–24]. However, in 2014, the CLIA method was widely accepted and applied for progesterone measurement [25–27].

Table-1: Mean, SD, 95% CI, Min, and Max value for serum progesterone concentration with quantification using the CMIA and Vcheck® for estimates during the early proestrus, LH peak, pre-ovulation, ovulation, post-ovulation and all periods of the bitch.

<table>
<thead>
<tr>
<th>Period</th>
<th>CMIA</th>
<th>Vcheck®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>95% CI</td>
</tr>
<tr>
<td>Proestrus</td>
<td>1.24 ± 0.37</td>
<td>1.11–1.38</td>
</tr>
<tr>
<td>LH peak</td>
<td>2.65 ± 0.36</td>
<td>2.35–2.95</td>
</tr>
<tr>
<td>Pre-ovulation</td>
<td>3.71 ± 0.52</td>
<td>3.31–4.10</td>
</tr>
<tr>
<td>Ovulation</td>
<td>6.79 ± 1.32</td>
<td>6.31–7.27</td>
</tr>
<tr>
<td>All period</td>
<td>5.75 ± 5.79</td>
<td>4.57–6.94</td>
</tr>
</tbody>
</table>

Table-2: Measure of agreement: Concordance correlation coefficient, Pearsons’ correlation coefficient, ant bias correction factor.

<table>
<thead>
<tr>
<th>95% limits of agreement (Bland and Altman)</th>
<th>Lin’s concordance correlation coefficient</th>
<th>95% confidence interval</th>
<th>Pearsons’ correlation coefficient</th>
<th>Bias correction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average difference</td>
<td>Lower</td>
<td>Upper</td>
<td>0.877</td>
<td>Lower</td>
</tr>
<tr>
<td>1.26</td>
<td>3.16</td>
<td>5.68</td>
<td>0.877</td>
<td>0.834</td>
</tr>
</tbody>
</table>
CMIA, an advanced adaptation of CLIA, is currently employed by veterinary reference laboratories in Thailand [18]. It’s important to note that both CLIA and CMIM have drawbacks, as they require several hours to several days for processing, depending on the laboratory’s location. On the other hand, point-of-care or in-house measurement of progesterone is gaining popularity among veterinary practitioners because of its simplicity, convenience, and rapid delivery.

In this study, serum handling differed based on the analyzers used: half of the harvested serum underwent analysis with the CMIA analyzer, whereas the remaining half was frozen and stored at −20°C before Vcheck® testing. This divergence addressed specific aspects of the study’s objectives. Bolelli et al. [28] reported a decrease in progesterone levels after long-term storage at −70°C, potentially attributed to molecular modification or interference by the cryotube material. This insight is pertinent to our study because it raises the question of long-term storage effects on analytes. Conversely, Key et al. [29] reported apparently normal progesterone levels in sera stored at −20°C for up to 7 years. This finding contrasts with Bolelli et al.’s observations and adds complexity to our understanding of storage effects on progesterone levels. Volkman [27] investigated the effects of anticoagulants, storage time, temperature, and assay methods on blood progesterone concentrations in dogs, expanding our understanding of the variables influencing progesterone measurements. The study’s outcomes yielded crucial insights: (i) RIA measurements indicated significantly higher sP4 concentration than CLIA; (ii) initial refrigeration of whole blood within 2 h post-collection notably decreased serum progesterone concentration; (iii) storage temperature of whole blood for up to 5 h had no apparent impact on progesterone concentration in heparinized plasma; and (iv) refrigeration of whole, clotted blood had no effect on serum progesterone concentration, given samples were at room temperature (25°C) for the first 2 h after collection. These findings are consistent with the main focus of this study on understanding the factors influencing blood progesterone levels in canines. To improve coherence, the findings of this study directly inform the primary focus on understanding the effects of various factors on blood progesterone levels in canine subjects. Therefore, in this study, all samples were promptly separated within 2 h after whole blood collection to obtain serum, ensuring accurate analysis. To maintain sample integrity and stability before Vcheck® testing, rapid separation and subsequent storage at −20°C were conducted to minimize any potential degradation of analytes.

The rapid measurement of serum progesterone levels is of the utmost importance as it allows accurate diagnoses and informed clinical decisions, especially in situations such as breeding or the careful planning of cesarean section. In view of the diverse requirements for precise diagnostics and well-considered decision-making, veterinarians consider commercial immunological analyzers indispensable. Several researchers have reported the accuracy of other commercial point-of-care immunologic analyzers compared with gold-standard methods, such as RIA, LC-MS, and computed tomography (CLIA) [5, 18–20, 30]. However, the present study is the first to report the use of the Vcheck® analyzer, providing novel insights into its accuracy and application. In conclusion, this study confirms the reliability of the Vcheck® analyzer for the swift measurement of serum progesterone levels in bitches. The Pearson’s correlation coefficient between Vcheck® and CMIA exceeded the significance threshold of 0.09 (Table 2). These results undeniably indicate a strong and significant relationship [31]. This discovery offers compelling proof of the inherent accuracy of serum progesterone concentration measurements obtained using Vcheck®. Table-1 presents the mean, SD, 95% CI, and range of serum progesterone concentration. This allows for a detailed comparison between Vcheck® and CMIA in estimating these values across different phases of the female dog’s reproductive cycle, including proestrus, LH peak, pre-ovulation, and ovulation and post-ovulation. This table exhibits distinct sets of values, designated with I lowercase letters and meticulously organized in rows corresponding to the Vcheck® analyzer. Each value marked with
Table 3: Reference or guideline for progesterone interpretation using Vcheck® in heat or apparent reproductively quiescent bitches.

<table>
<thead>
<tr>
<th>Progesterone by CMIA (ng/mL)</th>
<th>Progesterone by Vcheck® (ng/mL)</th>
<th>Likely events</th>
<th>Suggestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>1.00–2.81 (1.16–1.56)</td>
<td>Anestrus, proestrus, and pre-LH surge LH surge</td>
<td>• Confirm heat or proestrus by physical examination or vaginal cytology.</td>
</tr>
<tr>
<td>2.00–2.99</td>
<td>1.00–2.73 (1.42–2.33)</td>
<td>Post-LH surge, pre-ovulation</td>
<td>• Retest in 3 days</td>
</tr>
<tr>
<td>3.00–4.99</td>
<td>1.77–7.01 (2.17–4.53)</td>
<td>At or near ovulation</td>
<td>• Retest in 2 days to confirm continued rise in progesterone.</td>
</tr>
<tr>
<td>5.00–9.99</td>
<td>1.00–10.60 (4.47–6.25)</td>
<td>Post-ovulation, Oocyte maturation, in fertilizable period</td>
<td>• Aim for breeding 4–7 days.</td>
</tr>
<tr>
<td>&gt;10</td>
<td>5.77–21.27 (10.26–14.85)</td>
<td></td>
<td>• Retest in 1–2 days to confirm continued rise in progesterone.</td>
</tr>
</tbody>
</table>

Min=minimum, Max=maximum, CMIA=Chemiluminescence microparticle immunoassay

The aim of the present study was to confirm the validity of the Vcheck® analyzer for measuring serum progesterone levels in bitches, which is a crucial factor in assessing their reproductive status. Serum progesterone concentrations usually show a sharp increase (spike) at ovulation following a slow increase in proestrus, peak LH, and pre-ovulation. This pattern is pivotal in understanding the reproductive cycle. Moreover, this study paves the way for future research endeavors, especially exploring specific enhancements in the assessment of serum progesterone. These improvements strengthen veterinary practices and advance reproductive management by providing more accurate and reliable tools. In accordance with this objective, the guidelines presented here have been carefully formulated. These guidelines were devised by aligning with established CMIA guidelines and adapting them through the incorporation of range and 95% CI derived from each set of Vcheck® results (Table-3).

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Conclusion

The Vcheck® analyzer provides a rapid assessment of serum progesterone concentration in bitches, with results comparable to those obtained using the CMIA method. However, when considering the use of the Vcheck® analyzer, it is advisable to interpret the results carefully and follow the interpretation guidelines given in Table-3. In conclusion, Vcheck® provides a reliable and convenient option for veterinary practitioners to measure canine progesterone levels in a clinical/hospital setting.

Authors’ contributions

SW, TS, SA, WP, and SR: Study conception and design, conducted the study, and analyzed the
data. SW and SR: Sample preparation. SW, TS, and SR: Drafted the manuscript. All authors have read, reviewed, and approved the final manuscript.

Acknowledgments

The present study received partial financial backing from Mahanakorn University of Technology (Thailand) and BestAgro Companion (Thailand), through the allocation of funds provided by the Veterinary Research Grant under Contract No. Immuno-002-2020. Furthermore, the authors extend their sincere appreciation to Bangkok R.I.A. Co., Ltd. (Thailand) for their valuable support in the field of CMIA. Finally, the authors are thankful for sample preparation assistants, especially Tanabadee Jinaphom, Wisan Waraakesiri, Chutarat Noonjui, Pattamawan Potjes, and Nadol Utsahapalun.

Competing Interests

The authors declare that this study received funding from BestAgro Companion (Thailand) and Bangkok R.I.A. Co., Ltd. (Thailand) to maintain transparency and financial support. It’s important to note that the funders were not involved in the study design, data collection, analysis, interpretation, or the writing of this article, nor in the decision to submit it for publication, ensuring the independence and integrity of the research process. Additionally, the authors declare that they have no competing interests, further underscoring the transparency and credibility of this study.

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