Calcium, parathyroid hormone, oxytocin and pH profiles in the whelping bitch


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Abstract

Despite the high prevalence of primary uterine inertia in whelping bitches, the underlying pathogenesis remains unclear. The objectives were to i) determine serum concentrations of total calcium, ionized calcium (iCa), parathyroid hormone (PTH), and blood pH in normally whelping bitches throughout the peri-parturient period; and ii) investigate relationships among iCa, PTH, and acid-base status, and the role that they and oxytocin may have in the underlying pathogenesis of canine uterine inertia. Bitches were randomly selected from a population of German Shepherd Dog bitches with a history of uncomplicated parturition (Group 1; \( n = 10 \)), and from a population of Labrador bitches with a clinical history of an increased incidence of uterine inertia and stillbirths (Group 2; \( n = 20 \)). Jugular blood samples were collected daily from -4 d to the onset of whelping (\( t = 0 \) h), and then every 4 h until the last pup was born. Overall, bitches from Group 2 had higher mean ± SEM serum concentrations of PTH (4.72 ± 2.45 pmol/L, \( P < 0.001 \)), lower iCa (1.31 ± 0.08 pmol/L, \( P < 0.05 \)), and higher venous pH (7.41 ± 0.03, \( P < 0.005 \)) than bitches from Group 1 (2.9 ± 1.44 pmol/L, 1.38 ± 0.06 mmol/L, and 7.33 ± 0.02, respectively) during the periparturient period. However, there was no significant difference between Groups 1 and 2 for serum oxytocin concentrations during the periparturient period (45.5 ± 40 and 65.5 ± 82 pg/mL). We inferred that low iCa resulting from a rising pH and decreasing PTH during the periparturient period may have contributed to decreased uterine contractility and increased risk of stillbirths. Therefore, manipulating the cationic/anionic difference in diets of pregnant bitches, similar to the bovine model for hypocalcaemia, may reduce the incidence of stillbirths in the bitch.

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1. Introduction

Primary uterine inertia is a well recognized clinical complication in the parturient bitch, which is often resolved with slow intravenous administration of calcium gluconate [1–3], despite total serum calcium often being within the ‘normal reference range’ for non pregnant dogs (clinical observations and [4]). Resolution of dystocia caused by primary uterine inertia with the administration of calcium indicates its potential role in this disease. Oxytocin, alone or in association with calcium, has also been widely used in the treatment of suspected uterine inertia [2,3], despite equivocal evidence regarding its role in uterine inertia [5,6].
Indeed, oxytocin is difficult to measure and a ‘normal’ reference range has not been established for whelping bitches. However, resolution of some primary uterine inertia associated with dystocia with oxytocin indicates also its potential role in the pathogenesis of primary uterine inertia.

Unfortunately, the pathogenesis of either ‘complete’ or ‘partial’ uterine inertia in the bitch has not been studied in detail [1,2,4,7]. If hypocalcaemia is involved, there are, to the authors’ knowledge, no reports on the relationship between blood calcium and parathyroid hormone (PTH) concentrations, the effect that acid-base status has on these end points during the peripartum period in the bitch, and their effects on the incidence of uterine inertia.

During the peripartal period, the bitch has an increased demand for calcium resulting from skeletal ossification of the fetus, initiation of lactation, and increased activity of myometrial muscle. Forceful uterine contractions required for the expulsion of pups during whelping is dependent on the influx of free, ionized calcium into the myometrial cells [2,8]. However, free calcium availability may be restricted during this period of high demand due to transient inappetence, which many bitches experience on the day of whelping [2], or it may be further exacerbated by an acute respiratory alkalosis caused by panting, anxiety, fear, and pain (Henderson-Hasselbach Equation [9]) occurring in many bitches during whelping [10]. Increased protein binding of serum calcium [11] associated with a reduction in the amount and delay in the secretion of PTH in response to demand [12] may then occur, resulting in subsequent hypocalcaemia. This may then result in uterine inertia with delayed delivery, intrapartal hypoxia, and eventually birth of “stillborn” pups [1,13]. A pre-existing parathyroid gland atrophy has also been implicated in the underlying pathogenesis of eclampsia [3,14], and may be a contributing factor in the development of primary uterine inertia.

Two reports have documented the plasma concentrations of calcium and PTH in pregnant and lactating bitches [15,16]. However, in one study, only two bitches were used, only total non-ionized calcium was measured, and concentrations were only determined only once weekly during pregnancy and lactation [16]. The second study measured total and ionized serum calcium concentrations on the day prior to and the day of parturition, but not throughout whelping [15]. Furthermore, the latter authors did not investigate the relationship of calcium and PTH during whelping, nor their relationship with dystocia.

To better understand the pathogenesis of primary uterine inertia and thus eventually implement prevention and management strategies to reduce the incidence of this disease, determination of reference ranges for calcium (total and ionised), PTH, and oxytocin concentrations in bitches during the peri-parturient period is required.

The objectives of this study were to: i) determine the serum concentrations of total calcium, ionized calcium (iCa), parathyroid hormone (PTH), and oxytocin in 10 normally whelping bitches during the periparturient period; ii) investigate the relationship between blood calcium (iCa), PTH, and acid-base status (blood pH); and iii) investigate the potential role of oxytocin during the days around whelping in a subset of bitches randomly selected from a much larger population of breeding bitches, either with a clinical history of uncomplicated parturition (Control; Group 1), or with a clinical history of an increased incidence of uterine inertia and stillbirths (Group 2).

2. Materials and methods

2.1. Animals

2.1.1. Historical colony data

Group 1: Bitches in Group 1 (n = 10) were randomly selected from a research colony of German Shepherd Dog (GSD) bitches, aged from 2 to 7 yr, which were housed at The College of Veterinary Medicine, Cornell University, Ithaca, NY, USA. This colony had a history of low stillbirth prevalence (<1% over 5 yr).

Group 2: Bitches in Group 2 (n = 20) were randomly selected from a colony of Labrador bitches, aged from 2 to 7 yr, located at “The Guiding Eyes”, NY, USA. This colony had a clinical history of a high prevalence of stillborn pups due to primary uterine inertia (7%) over a 2 yr period. Other causes of stillbirths were ruled out, based on clinical examinations of both bitches and stillborn pups. Furthermore, necropsy and histopathology were performed on each stillborn pup to detect underlying infectious (e.g., bacterial, viral, protozoal) causes.

The historical stillbirth rates differed between the two colonies (P < 0.05). Confidentiality agreements prevent us from publishing raw historical stillbirth data.

2.1.2. Experimental animals

Procedures described herein were approved by the Institutional Animal Care and Use Committee at Cornell University.

Bitches from both Groups 1 and 2 were fed a balanced, pelleted commercial diet (Purina Pro Plan...
Adult Large Breed Formula®), Nestle Purina Petcare Company, St. Louis, MO, USA) ad libitum until 2 wk prior to the predicted whelping date. Thereafter, bitches were fed a combination of formulated dry food for puppies (Purina Puppy Chow®), supplemented daily with half a can of gastroenteric canine formula (Purina EN®) with the following content (g/can): protein, 32; fat, 14.42; carbohydrate, 51.24; fiber, 0.96; calcium, 0.92; phosphorus, 0.57; potassium, 0.64; sodium, 0.39; chloride, 0.82; and magnesium, 0.07).

2.2. Experimental design

2.2.1. Clinical assessments

Bitches from both colonies were under 24 h surveillance, starting from 7 d prior to the expected whelping date (65 ± 1 d after the estimated LH surge) until the last pup was born. The LH peak (Day 0) was estimated from serum progesterone concentration, as demonstrated by Concannon et al., 1989 [17], and was based on the day progesterone significantly increased and was ~2 ng/mL. The viability of each pup was determined immediately after birth, so that the number of pups born dead (i.e., stillborn pups) was accurately recorded. A thorough examination of each newborn pup was not carried out until 1–2 h after the last pup was born.

2.2.2. Collection, handling, and processing of blood samples

Jugular blood samples were collected daily, starting 4 d prior to the predicted whelping date, and then every 4 h from the onset to the end of whelping (time 0 = onset of Stage 2 of labour as determined by visible abdominal straining, engagement of the fetus into the pelvic canal, movement through the dilated cervix and along the birth canal, until ultimately the fetus was expelled with its associated fetal membranes and fluid along the birth canal, until ultimately the fetus was expelled with its associated fetal membranes and fluid until the last pup was born. The LH peak (Day 0) was estimated from serum progesterone concentration, as demonstrated by Concannon et al., 1989 [17], and was based on the day progesterone significantly increased and was ~2 ng/mL. The viability of each pup was determined immediately after birth, so that the number of pups born dead (i.e., stillborn pups) was accurately recorded. A thorough examination of each newborn pup was not carried out until 1–2 h after the last pup was born.

2.2.3. Analysis of blood and serum samples

Total serum calcium was determined by the Clinical Pathology Laboratory at Cornell University, with an automated chemistry analyser (Hitachi 917, Roche), using an o-cresolphthalein complexone method. The ionized calcium concentration and pH of each heparinised venous sample were also determined within 11 wk, at The Michigan State University Veterinary Clinical Pathology Laboratory, East Lansing, MI, USA, using an ion selective electrode on the Nova 8 analyser [20]. Using an established standard curve, serum ionised calcium concentrations were adjusted to a standard pH of 7.4 [20].

The parathyroid hormone (PTH) concentration of each serum sample was measured at Michigan State University Pathology Laboratory, using a heterologous radioimmunoassay (RIA) for intact human PTH (Nichols, San Juan Capistrano, CA, USA). This assay has been validated for dog serum [21]. To reduce interassay variation, all samples were analysed in one single batch at 11 wk after collection.

The concentration of oxytocin in each serum sample was analysed at the University of Florida, Endocrine laboratory of the Veterinary College, Gainesville, FL, USA, using an Oxytocin Enzyme immunoassay (EIA) developed by Assay Designs and validated for dog serum (Oxytocin Enzyme Immunoassay, Kit No 900-153; Assay Designs Ann Arbor, MI, USA). To improve sensitivity and reduce serum interference, all serum samples were first extracted using C18 micro-columns (Thermo Fisher Electron Corp Hypersep C18 500 mg column ref 60108-305, Pittsburg PA, USA) as follows. Briefly, 500 μL of serum was diluted v/v with 0.1% TFA in water and centrifuged at 17000 × g for 15 min at 4 °C. After centrifugation, the supernatant was collected and injected on the conditioned HyperSep column. Conditioning of the columns included, injection of 1 mL of 100% acetonitrile and washing with 10 mL of 0.1% TFA in water. After conditioning and injection of the sample, the column was washed with 10 mL of TFA 0.1% in water, and the oxytocin was
eluted using 3 mL of 60% acetonitrile and 40% of 0.1% TFA in water (Fisher, Alachua, FL, USA). Samples were collected and evaporated to dryness in a vacuum centrifuge at room temperature. (Eppendorf Vacufuge Concentrator, model 5301, Fisher). Percentage recovery as tested using male canine serum added with 200 ng/mL of oxytocin was 95.2% ± 2 (n = 10) and linear over the range 20 to 400 ng/mL. All samples were then re-extended in PBS pH 7.2 at the same volume as initial before being submitted to the assay as described by the company. The intra-assay (n = 10) and inter-assay (n = 5) coefficients of variation were 7.2 and 10.7 respectively for a sample of 80 pg/mL, and were 9.1 and 14.5 for a sample of 17.5 pg/mL. The sensitivity limit of the system at two standard deviations from the zero along the standard curve was 11.7 pg/mL. All samples were assayed in duplicate (Verstegen J. and Burrows J., personal communication).

2.3. Statistical analyses

Data for calcium concentrations (ionized and total), PTH, venous pH, pCO₂, glucose and oxytocin for each sampling time was analysed with repeated measures ANOVA, for main effects of Group, time, and their interaction. Post-hoc multiple pair wise comparisons were performed using Tukey’s test. All analyses were performed using SigmaPlot Version 11.0 (2008, SPSS Inc., Chicago, IL, USA) and percent recovery as tested using male canine serum added in a vacuum centrifuge at room temperature. (Eppendorf Vacufuge Concentrator, model 5301, Fisher).

3. Results

3.1. Mean concentration of PTH, iCa, total calcium, and pH in bitches from Group 1

Mean concentrations of serum PTH, iCa, total calcium, and venous pH during the periparturient period of bitches from the Control group of experimental bitches (Group 1) are shown (Table 1).

<table>
<thead>
<tr>
<th>Serum</th>
<th>Day</th>
<th>Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-4</td>
<td>-3</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>5.05 ± 0.21</td>
<td>4.07 ± 2.66</td>
</tr>
<tr>
<td>Ionised calcium ** (mmol/L)</td>
<td>1.38 ± 0.04</td>
<td>1.36 ± 0.05</td>
</tr>
<tr>
<td>Venous pH</td>
<td>7.36 ± 0.04</td>
<td>7.36 ± 0.02</td>
</tr>
<tr>
<td>Total calcium (mg/dL)</td>
<td>9.80 ± 0.57</td>
<td>9.79 ± 0.84</td>
</tr>
</tbody>
</table>

Time 0 h = onset of Stage II of whelping, as determined by forceful abdominal contractions and expulsion of the first fetus [2].

** adjusted to pH 7.4.

3.2. Relationship between iCa, PTH, and acid-base status during the periparturient period of bitches from Groups 1 and 2

Only the main effect of Group had a significant effect on the dependent variables. There was no significant effect of time, or Group by time interaction, except for pCO₂, where time relative to whelping was significant.

Overall, bitches from Group 2 had a higher mean PTH (4.72 ± 2.45; P < 0.001), lower mean serum iCa concentration (1.31 ± 0.08; P < 0.05), and higher mean venous pH (7.41 ± 0.03; P < 0.005) than bitches from Group 1 (2.9 ± 1.44, 1.38 ± 0.06, and 7.33 ± 0.02, respectively), during the periparturient period.

Serum iCa concentrations decreased at the onset of whelping for bitches from both Groups 1 and 2. However, bitches from Group 1 had increased blood iCa concentrations after the onset of Stage 2 of labour, compared to bitches from Group 2 (Fig. 1A; P < 0.05).

Although not significant, there was a decrease in serum PTH concentrations in bitches from Group 1 at the onset of whelping (time = 0 h; Fig. 1B) in response to the increased calcium requirements at this time. This result was inversely reflected by the rapid rise in iCa concentrations in bitches from Group 1 during whelping (Fig. 1A). Furthermore, bitches from Group 2 had no change in PTH concentrations during the periparturient period (Fig. 1B; P > 0.05). This result was also inversely reflected by the continuously lower iCa concentrations in these bitches until 12 h after the onset of whelping (time = 0 h; Fig. 1A).

Importantly, bitches from Group 1 had a slower and gradual rise in venous pH after the onset of Stage 2 of labour (time = 0 h) compared to bitches from Group 2 that experienced a sharp and steep rise in venous pH at the onset of whelping (time = 0 h; Fig. 2A; P < 0.05). Interestingly, bitches from Group 2 had a higher venous pH than bitches from Group 1 at 3 d prior to the onset of whelping (P < 0.05).
Furthermore, venous pCO₂, the respiratory component of acid-base regulation, was lower for bitches from both groups (32.2 and 32.4 mmHg respectively) than the normal reference range for dogs (35–38 mm Hg) from the onset of whelping (time = 0 h) until the end of whelping (Fig. 2B; $P < 0.05$). Similarly, observation of increased respiratory effort/panting at the onset of whelping occurred as well, but this was only a subjective assessment that could not be statistically analysed.

### 3.3. Measurement of iCa by the iSTAT analyser compared to laboratory determination

Serum iCa concentrations determined by the iSTAT analyser for bitches from Groups 1 and 2 were similar (1.26 ± 0.10 and 1.29 ± 0.06 nmol/L, $P > 0.05$). However, analysis by the Nova 8 laboratory analyser (Michigan State University Veterinary Clinical Pathology Laboratory) detected a difference in iCa concentrations in bitches from Group 1 (1.38 ± 0.056 nmol/L) compared to bitches from Group 2 (1.31 ± 0.081 nmol/L; $P < 0.001$).

### 3.4. Measurement of blood total calcium, glucose, and oxytocin in bitches from groups 1 and 2 during the periparturient period

Bitches from Groups 1 and 2 had similar $P > 0.05$ mean serum concentrations of total calcium (Fig. 1C; 9.9 ± 1.0 and 9.2 ± 0.7 mg/dl respectively), glucose (108.3 ± 7.6 and 115.1 ± 13.6 mg/dL), and oxytocin (45.5 ± 40 and 65.5 ± 82 pg/mL) throughout the
periparturient period. Furthermore, there was no change in serum concentrations of total calcium (Fig. 1C), glucose or oxytocin throughout the periparturient period \( (P > 0.05) \).

3.5. Interpup interval, average number of pups born, average whelping duration, and stillbirth rate

The average number of pups born for bitches from Groups 1 and 2 was 7.2 and 6.5 pups, respectively. The mean time taken and whelping duration from the onset of Stage 2 of labor until the last pup was born was 7.9 ± 3.6 h (range, 2.5–12) and 6.1 ± 2.7 h (range, 4–10.5) respectively \( (P > 0.05) \). There was no difference in the time taken to whelp each pup in bitches from Groups 1 and 2 (1.10 and 1.08 h/pup, \( P > 0.05 \)). The number of pups in each litter had no effect on either the duration of whelping or number of stillborn pups. Furthermore, there was no difference in the stillbirth rate between bitches from Groups 1 and 2 (0.6 and 0.2 pups, \( P > 0.05 \)) and none of the bitches sampled for this study were diagnosed or treated for uterine inertia.

4. Discussion

Despite the small number of bitches in the Control group, this is apparently the first report of serum calcium (total and ionised) and PTH concentrations in normally whelping bitches (Group 1; Control) during late gestation and parturition. Furthermore, this is apparently the first report investigating the changes and interactions that occur in iCa, PTH, and pH during the immediate periparturient period of normally whelping bitches (Group 1; Control).

The total calcium results in this study supported previous reports of the inaccuracy in measuring this form of calcium for clinical diagnosis of uterine inertia \([1,4,20]\). Total calcium is composed of ionised, complexed, and protein bound fractions and therefore is an insensitive indicator of the plasma concentration of the physiologically and biologically active form of calcium \([14]\). Furthermore, the significant difference in iCa concentrations in the same blood samples measured by the iSTAT analyser compared to adjusted laboratory analysis emphasized the importance of exercising caution when interpreting results from dog-side analysers such as the i-STAT machine for the diagnosis of hypocalcemia and uterine inertia in the whelping bitch \([20]\).

Hypoglycaemia has also been implicated as a cause of primary uterine inertia, although mainly in small breeds \([6]\). However, similar to the clinical findings in 27 bitches with clinical uterine inertia reported by Bergstrom et al. \([22]\), the bitches in this study did not have significantly low (below the normal reference range for dogs, 60–115 mg/dL) blood glucose concentrations throughout the peri-parturient period.

The incidence of clinical uterine inertia, stillbirths, and inter-pup intervals was not significantly different between the bitches sampled from each population/ colony of bitches with or without a clinical history of increased stillbirth incidence. The objective of this study was not to present a case report, but rather to explore the underlying pathogenesis of uterine inertia at a subclinical level in a large population of historically documented (via stillbirth rate and clinical uterine inertia incidence data) susceptible Labrador bitches. Measurement of iCa, PTH, and oxytocin in nonpregnant bitches from both these colonies was not conducted, but may contribute to greater understanding of the pathogenesis of uterine inertia in these two breeds.

Based on this study, we inferred that ultimately decreased availability of the physiologically active form of calcium played an important role in the underlying cause of primary (complete or partial) uterine inertia and the subsequent increased incidence of stillbirths. Although no clinical cases of uterine inertia were observed during this study, bitches from Group 2 had significantly lower mean ionised calcium concentrations than bitches from Group 1, despite a significantly higher mean PTH concentration. It appears that a rising pH (alkalosis) and declining pCO2 at the onset of whelping in bitches from Group 2 lead to a subclinical respiratory alkalosis or mixed acid base disorder \([9]\), which in turn may have resulted in decreased tissue (bone, intestinal and renal) responsiveness to PTH, as reported by Lopez et al., 2003 \([12]\), thus resulting in a transient decrease in blood calcium concentrations, decreased uterine contractility and increased risk of stillbirth incidence. The findings in this study are similar to the clinical model for hypocalcaemia or “milk fever” in the pregnant dairy cow reported by Goff et al., 2004 \([22]\). In the bovine model, metabolic alkalosis is demonstrated to reduced tissue responsiveness to PTH thus preventing cows from maintaining calcium homeostasis through bone calcium reabsorption and renal production of 1, 25-dihydroxyvitamin D3 \([3]\) at the onset of lactation resulting in clinical hypocalcaemia \([22]\).

Manipulation of the dietary cation-anion (DCAD) balance has been used effectively to prevent subclinical hypocalcaemia in periparturient dairy cows \([22–25]\). The supplementation of dairy cow diets prior to calving with anions (chloride, sulfate) induces a mild metabolic acidosis that, in turn, increases plasma calcium.
concentration through an increased responsiveness of tissues to PTH [22,25,26]. Dietary manipulation of the cationic/anionic difference in diets formulated for pregnant bitches may also be a potential preventative treatment to aid in reducing the incidence of stillbirths in the bitch by lowering arterial blood pH. In a recent study carried out by Lopez et al., 2002 [27], artificial induction of acute acidosis not only increased PTH secretion in the presence of normal ionised calcium levels, but enhanced PTH secretion in response to hypocalcaemia. Therefore, manipulation of diets for pregnant bitches, especially those with a previous history of developing hypocalcaemia and primary uterine inertia during whelping, may be beneficial in reducing the incidence of stillbirths. It was interesting that bitches from Group 2 had a higher venous pH 3 d prior to the onset of whelping, compared to bitches from Group 1. Determination of the venous pH throughout the gestational period of bitches from Group 2 is required to determine the relevance of this finding and that indeed these bitches are prone to alkalosis during pregnancy and therefore benefit from dietary manipulation intervention.

Bergstrom et al. (2006) [4] recently reported 27 clinical cases of canine uterine inertia and reported that they all presented with low oxytocin concentrations (30–35 pmol/L). However, Klarenbeek et al., (2007) [6] examined profiles of plasma oxytocin concentrations during late pregnancy and throughout the expulsive stage of parturition in eight normally whelping bitches and demonstrated significant variation in oxytocin secretion in normally whelping bitches, not only within and between individuals, but throughout the periparturient period, in particular during the “expulsive phase” (range 10–117 pg/mL), when most single clinical measurements would be carried out. The large variation in the oxytocin concentrations taken at each time point throughout whelping for bitches from both groups in this study supported the observations reported by Klarenbeek et al., (2007) [6]. Furthermore, they found that there was no correlation between oxytocin peaks and expulsion of pups during whelping. Thus determination of clinically significant levels of oxytocin at any one time point during whelping or determining a “normal” reference range may be very difficult. The findings from this study, as well as the studies carried out by Olsson et al., (2003) [5] and Klarebeek et al., (2007) [6] highlighted the multifactorial nature of the pathogenesis of uterine inertia in the bitch and the need for more detailed investigations into the role of oxytocin, calcium, and their respective uterine receptors in the control of uterine contractions.

Although primary uterine inertia is not a known hereditary disorder, it is common in clinical practice to see related groups of bitches suffer from a higher prevalence of uterine inertia (personal observations). Bitches in Group 2 were of a single breed (Labrador), from the same breeding colony, and in some cases closely related; all may have experienced a level of subclinical changes which may pre-dispose this breed to uterine inertia. Perhaps in the future there will be genetic markers for uterine inertia/hypocalcaemia in bitches.

Although uterine inertia is one of the most common causes of dystocia in the bitch [5], there have been apparently no reports on relationships among blood calcium and PTH concentrations and acid-base status during the peri-parturient period, or their role in the pathogenesis of uterine inertia in the bitch. This study was apparently the first to report serum concentrations of calcium and PTH, not only in whelping bitches during late gestation and parturition, but in bitches from a population with a clinical history of uterine inertia and an associated increased stillbirth rate, thus providing an insight into subclinical changes in calcium, PTH and pH concentrations that may pre-dispose bitches to developing uterine inertia. It is expected that future investigations will contribute much needed information, not only regarding the pathogenesis of this disorder, but also to facilitate formulation of diets for pregnant bitches that will optimize calcium homeostasis in the parturient bitch.

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References


