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—Original Article—

The effect of metritis and subclinical hypocalcemia on uterine involution in dairy cows evaluated by sonomicrometry

Maike HEPPELMANN[1)], Karoline KRACH[1)], Lars KRUEGER[1)], Philipp BENZ[1)], Kathrin HERZOG[1)], Marion PIECHOTTA[1)], Martina HOEDEMAKER[1)] and Heinrich BOLLWEIN[2)]

[1)]*Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, 30173 Hannover, Germany*
[2)]*Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, CH-8057 Zurich, Switzerland*

Abstract. The objective of this study was to examine the effects of metritis and subclinical hypocalcemia on reduction of uterine size in dairy cows using ultrasonography and sonomicrometry. Four piezoelectric crystals were implanted via laparotomy into the myometrium of the pregnant uterine horn of 12 pluriparous Holstein Friesian cows 3 weeks before the calculated calving date. Sonometric measurements were conducted daily from 2 days before parturition (= Day 0) until Day 14 after calving and then every other day until Day 28. Distances between adjacent crystals were expressed in relation to reference values obtained before calving. The diameter of the formerly pregnant uterine horn was measured using transrectal B-Mode sonography starting on Day 10. Cows were retrospectively divided into the following groups: cows without metritis (M–; n = 7), cows with metritis (M+; n = 5), cows with normocalcemia (SH–; Ca > 2.0 mmol/l on Days 1 to 3; n = 5) and cows with subclinical hypocalcemia (SH+; Ca < 2.0 mmol/l in at least one sample between Days 1 and 3; n = 7). Metritis did not affect ($P > 0.05$) sonometric measurements, but the diameter of the formerly pregnant horn was larger ($P \leq 0.05$) between Days 15 and 21 in M+ cows than in M– cows. Reduction in uterine length in hypocalcemic cows was delayed ($P \leq 0.05$) between Days 8 and 21 compared with normocalcemic cows, but the uterine horn diameter was not related to calcium status. In conclusion, both diseases affected reduction of uterine size until Day 28. Cows with metritis had a larger uterine diameter, possibly attributable to accumulation of lochia, and cows with subclinical hypocalcemia had delayed reduction of uterine length, presumably related to reduction of myometrial contractility.

Key words: Calcium, Puerperium, Sonography

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Uterine involution occurs in the puerperal period and is a precondition for subsequent conception [1]. The reduction of uterine size is a major part of involution and is largely completed by 25 to 30 days postpartum [2, 3]. Myometrial contractions and the discharge of lochia in the first days after calving are of paramount importance for the decrease of uterine size [1, 4, 5]. Uterine involution can be affected by calving-related disorders and puerperal disease. Retained fetal membranes and metritis delay the return of the uterus to normal size [2, 3, 6, 7], but very little is known about the pathophysiology of puerperal uterine diseases [8]. Hypocalcemia reduces smooth muscle contraction [9] and has been associated with decreased myometrial contractility [10, 11]. Cows with a history of milk fever in the same lactation have greater uterine horn diameters between 15 and 32 days postpartum [12].

Monitoring of uterine involution can be achieved noninvasively using simple techniques such as transrectal palpation and transrectal B-Mode sonography [13, 14], but these procedures do not allow for assessment of the entire uterus in the first days after calving [15]. Experimental, invasive techniques including intrauterine pressure measurement [16, 17], electromyography [18, 19] and measurement using strain gauge transducers [20] cannot be used to monitor the reduction of uterine size during involution. Sonomicrometry is an objective technique used to measure distances between piezoelectric crystals based on the time an ultrasound signal requires to travel between a transmitter and a receiver [21]. Sonomicrometry has been used experimentally for continuous measurement of cardiac and skeletal muscle contractility [22–24], and a technique using sonomicrometry was recently established to examine reduction of uterine size in dairy cows. In a study involving seven Holstein Friesian cows, sonomicrometry was found to be suitable for assessment of the reduction in uterine length in the puerperium, and currently, it is the only practical technique available for this purpose [25].

The objective of this study was to investigate the effect of metritis and subclinical hypocalcemia on reduction of uterine size via sonomicrometry in dairy cows.

Materials and Methods

Cows

The study was carried out at the Clinic for Cattle of the University of Veterinary Medicine, Hannover, Germany, between September

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Correspondence: M Heppelmann (e-mail: Maike.Heppelmann@tiho-hannover.de)

2009 and April 2011 and was approved and conducted in accordance with German legislation on animal rights and welfare (33.9-42502-04-09/1696). Twelve pluriparous Holstein Friesian cows (age 3.4 ± 0.4 yr [mean ± SD]; parity 2.3 ± 0.5; body weight 677 ± 56 kg; 305 day fat-corrected milk yield 7,752 ± 874 kg) were used. The cows were housed in straw-bedded tie stalls and fed hay, corn silage and concentrate. After parturition the animals were milked twice daily.

Study design

Three weeks before the calculated calving date, sonometric crystals were implanted into the uterine wall via laparotomy. Sonometric measurements were conducted twice weekly from 3 weeks to 2 days before calving (day of calving = Day 0). Thereafter, measurements were made daily until Day 14 postpartum followed by every other day until Day 28. For each cow, the sonometric data from 21 days were analyzed for a total of 252 days. Transrectal B-mode sonography of the reproductive tract was carried out beginning on Day 10 postpartum until Day 28. During the study period, the cows underwent a daily general examination, transrectal palpation of the reproductive organs and vaginal examination. Blood samples were taken at the time of the sonometric measurements beginning one day after calving. Cows were retrospectively divided into 4 groups based on the occurrence of metritis and serum calcium concentrations. Cows of group M+ had metritis, and cows of group M– did not; metritis was diagnosed by vaginal and transrectal examination according to Sheldon [26]. Cows with metritis were treated locally with tetracycline hydrochloride boluses (4000 mg, Tetra-Bol®, CP-Pharma Handelsgesellschaft, Burgdorf, Germany) every 2 to 3 days until two weeks after calving. Cows of group SH– had normal serum calcium concentrations (Ca > 2.0 mmol/l on Days 1 to 3), and cows of group SH+ had subclinical hypocalcemia (Ca < 2.0 mmol/l on at least one day from Day 1 to 3). Subclinical hypocalcemia was treated on the same day the diagnosis was made by means of subcutaneous administration of 250 ml of a solution containing calcium gluconate, calcium borogluconate, calcium hydroxide and magnesium chloride (Calcitat S50®, aniMedica, Senden-Bösensell, Germany), which provided 11.4 g of calcium.

Sonometric system, measurements and analysis

The sonomicrometry system (Sonometrics, London, ON, Canada), the surgery for implantation of the piezoelectric crystals and the sonometric measurements and analysis have been described in detail [25]. In brief, the sonomicrometry system consisted of 4 piezoelectric crystals connected to a sonomicrometer (TRX8), a channel selector box and a Sonometrics data acquisition computer. The sonomicrometer generated ultrasound signals in the piezoelectric crystals and converted the received analogue signals into digital signals for further analysis. The four piezoelectric crystals (1–4) were implanted via left-flank laparotomy into the myometrium at the greater curvature of the pregnant uterine horn in a longitudinal direction. Sonometric measurements were always made during a 4-h period from 0900 h to 1300 h. In addition to video recording of the animals, physical activities were recorded in a written log to identify artefacts caused by movements of the cows. The SonoSOFT software (Version 3.4.30 RC1, Sonometrics) was used for data acquisition and analysis. Only the data from adjacent crystals were used. Thus the three distances between crystals 1 and 2, 2 and 3 and 3 and 4 were analyzed. The data were filtered, artefacts caused by movements of the cows were eliminated and periods with poor signal quality were excluded from the data set.

B-mode sonography

Transrectal B-mode sonographic examination was conducted with a GE Logiq Book XP ultrasound machine (GE Medical Systems, Jiangsu, PR China) equipped with a linear transducer (type i739L, 4–10 MHz). A cross-sectional image of the formerly gravid uterine horn 2 cm cranial to the bifurcation was obtained. The diameter (cm) was calculated as the mean of the maximum height and width of the endometrium.

Blood samples

All cows were fitted with an indwelling jugular catheter (WVI Jugularis-Katheter, Walter Veterinaer-Instrumente, Baruth/Mark, Germany) on Day 1 after calving. Blood samples were collected into serum tubes and immediately placed on ice. After centrifugation (3,500 × *g*, 10 min at 4 C), the serum was harvested and stored at –20 C until analysis. The total serum calcium concentration was measured using a Cobas Mira biochemistry analyzer (intra-assay coefficient of variation, 2.53%; Hoffmann-La Roche & Co AG Diagnostika, Basel, Switzerland).

Statistical analysis

Statistical analysis was carried out using the Statistical Analysis System (version 9.3., SAS Institute, Cary, NC, USA). The sonometric distances recorded on one day between Days 8 and 3 before calving were used as reference values representing 100%, and the measured distances were expressed in relation to the reference values. The mean value of the 3 distances was calculated. The Shapiro-Wilk test revealed that the distribution of the residuals of the relative lengths of the inter-crystal distances and the uterine horn diameter were normal, and they were expressed as the mean and SD. The effects of group (M–/M+ and SH–/SH+) and time on variables were analyzed separately for each week postpartum using PROC MIXED. Group SH or M, time (day postpartum), dystocia and interactions between the SH and M groups, SH group and time, M group and time were considered fixed effects, and cow was specified as a random term. Time was considered a repeated measure, and the Bayesian-Schwarz criterion and Akaike information criterion were used to determine the optimal covariance structure matrix (variance components and toeplitz). Differences among groups were analyzed using the Scheffé test (PROC GLM). Differences were considered significant at $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered to reflect a trend toward significance.

Results

Five (42%) cows had grade 1 metritis (M+), and the remaining 7 cows were free of metritis (M–). The mean calcium concentration did not differ between groups M+ and M– ($P > 0.05$). Seven cows (58%) had serum calcium concentrations <2 mmol/l (SH+), and 5 had concentrations >2 mmol/l (SH–). Two cows of the SH– group and 3 cows of the SH+ group had metritis. Daily general examination showed no signs of periparturient paresis in SH+ cows.

The mean gestation length was 281.5 ± 8.2 days. Delivery of the calves was aided by slight manual traction in 5 cows (42%) and strong traction in 3 cows (25%), and these 5 cows were categorized as having dystocia. Four cows calved spontaneously. Three of the 12 calves were stillborn (25%). The mean birth weight of the calves was 48.9 ± 6.4 kg. Groups M+ and M– and groups SH+ and SH– did not differ with respect to these variables (P > 0.10).

Sonometric and sonographic findings

Sonometric data could not be collected on 3 (1.2%) of the 252 examination days because of technical problems with the personal computer. Of the remaining data sets, 8.9% could not be analyzed because of poor signal quality or artefacts. Sonometric measurements were affected by time from Day 8 to 28 (P ≤ 0.05; Figs. 1 and 2). Presence or absence of metritis had no effect on sonometric measurements (P > 0.10; Fig. 1). Between Days 8 and 14, SH+ cows had greater (P ≤ 0.05) relative uterine lengths and from Day 15 to 21, there was a trend (0.05 < P ≤ 0.10) toward a greater relative uterine lengths in SH+ cows compared with SH– cows. The interaction between SH group and time tended to affect (P ≤ 0.10) the relative uterine length between Days 1 and 7 (Fig. 2). Dystocia, the interaction between M group and time and the interaction between SH group and M group had no effect (P > 0.10) on sonometric measurements.

The diameter of the formerly pregnant uterine horn was affected by time from Day 10 to 28 (P ≤ 0.05; Figs. 3 and 4). Between Days 15 and 21, cows with metritis had a greater (P ≤ 0.05) uterine diameters than cows without metritis (Fig. 3), and the interaction between SH group and time tended to affect (0.05 < P ≤ 0.10) uterine diameter (Fig. 4). Dystocia, SH group and interaction between M group and time and between SH group and M group did not affect uterine diameter (P > 0.10).

Discussion

The occurrence of metritis and the occurrence of subclinical hypocalcemia both adversely affected reduction of uterine size during the puerperal period albeit in different ways. Metritis delayed reduction of the uterine diameter, and subclinical hypocalcemia delayed the reduction of the uterine length and diameter compared with healthy cows.

The classification of cows as normocalcemic or hypocalcemic was performed between Days 1 and 3 because the serum calcium concentrations were lowest at this time, with the nadir occurring on Day 2 [27]. Hypocalcemia occurs due to the large amount of calcium secreted in colostrum and the inability of the cow to mobilize enough calcium from bone and to increase intestinal absorption [9]. The cutoff serum calcium concentration of 2.0 mmol/l used to differentiate the two groups was based on a study by Horst *et al*. [28]. The observed prevalence of SH (58%) was slightly higher than the prevalences of 50% and 41% reported elsewhere [28, 29]. Although an additive effect of hypocalcemia and metritis seemed possible, there was no interaction between groups M and SH on sonometric and sonographic measurements. Therefore, the observed differences could clearly be attributed to group allocation.

As expected, uterine involution was delayed in cows with SH as evidenced by delayed reduction of the uterine length from Day 1 to 21 postpartum. This was in agreement with the notion that hypocalcemia reduces myometrial contractility [10]. The myometrium consists of an outer longitudinal muscle layer and an inner circular layer. The longitudinal uterine musculature is responsible for the reduction in length, and the circular layer constricts the uterine lumen during involution [30]. The uterine diameter measured sonographically differed between SH groups only from Day 1 to 7, suggesting that hypocalcemia has a more profound effect on the longitudinal muscle

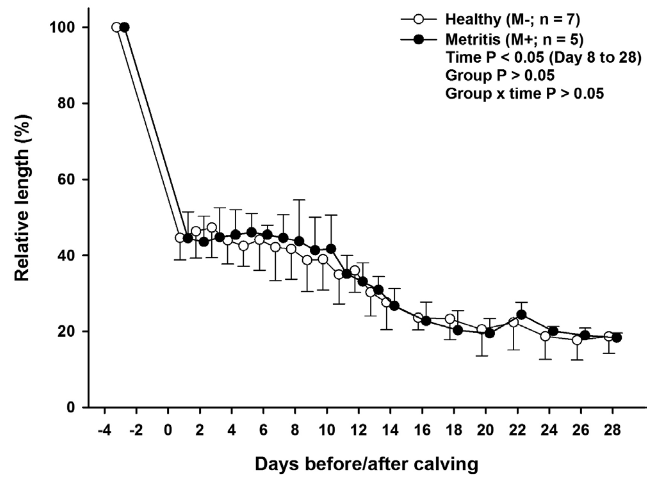

Fig. 1. Relative length (means ± SD) of the mean of three distances between sonometric crystals in 12 cows (n = 7, healthy; n = 5, metritis) in the first 28 days after calving. Relative length is expressed as a proportion of the baseline value taken 3 to 8 days before calving (Day 0 = day of calving).

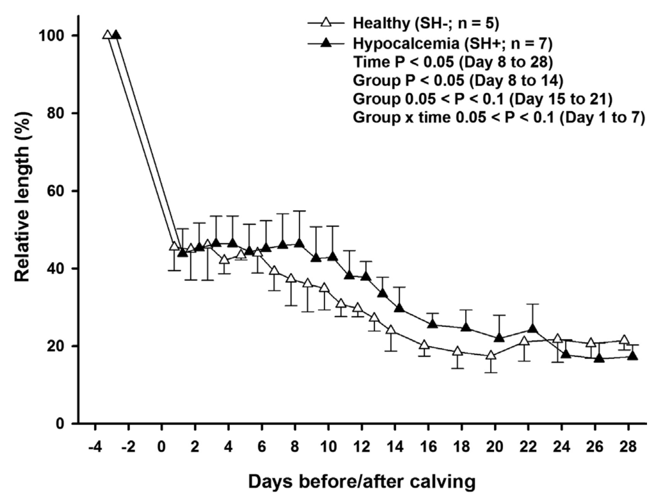

Fig. 2. Relative length (means ± SD) of the mean of three distances between sonometric crystals in 12 cows (n = 5, normocalcemia; n = 7, subclinical hypocalcemia) in the first 28 days after calving. Relative length is expressed as a proportion of the baseline values taken 3 to 8 days before calving (Day 0 = day of calving).

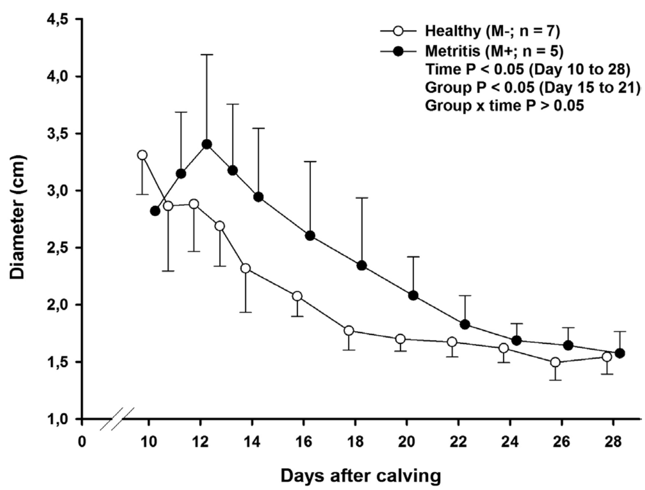

Fig. 3. Diameter of the formerly pregnant uterine horn (means ± SD) measured by transrectal B-mode sonography in 12 cows (n = 7, healthy; n = 5 metritis) between Days 10 and 28 after calving.

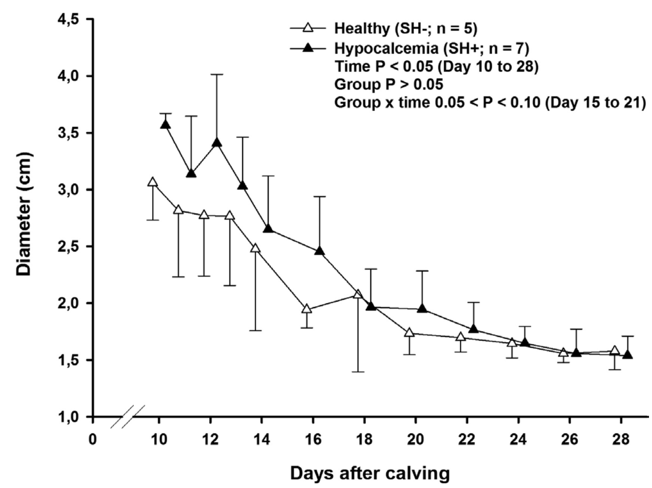

Fig. 4. Diameter of the formerly pregnant uterine horn (means ± SD) measured by transrectal B-mode sonography in 12 cows (n = 5, normocalcemia; n = 7, subclinical hypocalcemia) between Days 10 and 28.

layer than on the circular layer. The reason for this is not clear, but differential distribution of calcium channels in the myometrium is a possible explanation. In the myometrium of the rat, the expression of voltage-dependent calcium channels, which are the major venue for intracellular calcium availability, differs between the two muscle layers; there is a higher expression of L-type calcium channels in the longitudinal musculature of the pregnant rat uterus [31]. The fact that the adverse effect on involution occurred despite immediate treatment of affected cows with calcium is of great interest. This suggests that even short periods of hypocalcemia can adversely affect reduction of uterine size. Surprisingly, the adverse effect of hypocalcemia on gross uterine involution lasted much longer than the actual phase of hypocalcemia. Periparturient paresis did not occur in this study, and it can be assumed that this would have had a more severe effect on uterine contractility and involution than mere subclinical hypocalcemia. This stresses the importance of prevention of hypocalcemia.

We were surprised that the sonometric measurements were not affected by metritis, because uterine infection and inflammation can result in uterine atony [8]. Endotoxins released in association with uterine infection induce the production of PGE_2, which has a myorelaxant effect [32]. Cows with retained fetal membranes synthesize more PGE_2 than $PGF_{2\alpha}$ in placental tissue [33]. Cows with fetid sanguinopurulent lochia had higher PGE_2 concentrations than cows with purulent lochia [32]. Metritis in our study was relatively mild (grade 1) and possibly not severe enough to have a significant effect on uterine tone. Nevertheless, cows in group M+ had a larger diameter of the formerly pregnant uterine horn between Days 15 and 21 postpartum than cows without metritis. In addition to uterine contractility, it is likely that the accumulation of lochia contributed to an increase in diameter in affected cows [7, 8]. A larger-than-normal uterine diameter was also observed in the second week postpartum in cows with retained fetal membranes and/or metritis [2]. Although dystocia predisposes cows to puerperal uterine disease [8], it did not affect uterine involution in our study. Delayed uterine involution has a negative impact on reproductive performance [34, 35], but this was not examined in the present study.

In conclusion, metritis and subclinical hypocalcemia affected reduction of uterine size during the first 28 days after calving. Compared with healthy cows, cows with low-grade metritis had a larger uterine diameter when assessed sonographically, possibly attributable to accumulation of lochia, and cows with subclinical hypocalcemia had delayed reduction of uterine length, presumably related to reduction of myometrial contractility compared with normocalcemic cows. The fact that these effects occurred despite treatment of metritis and hypocalcemia stresses the importance of prevention of these disorders. Because of the small number of cows in this study, further investigations are necessary to confirm our findings.

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