Postpartum Pathologic Changes Associated With a Palpable Uterine Lumen in Dairy Cattle

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Abstract

Fifteen Holstein cows with a palpable uterine lumen (PUL) at first postpartum palpation were identified and each cow was matched with a normal cow for parity and stage of lactation. Blood was collected from each cow and the uterus cultured, flushed with lactated Ringer's solution and biopsied. Analysis of blood samples indicated that all cows were healthy. Bacteria were isolated from 14 of 15 PUL cows and 13 of 15 normal cows. Uterine flush analysis showed evidence of an inflammatory response in 5 PUL cows and 1 normal cow. Mean scores from microscopic examination of uterine biopsy specimens were greater ($P < 0.10$) in PUL cows, indicating increased pathologic changes in this group. In conclusion, cows with PUL at first postpartum rectal examination had increased pathologic changes suggestive of delayed uterine involution or permanent damage to the uterus.

Key words: palpable uterine lumen, dairy cattle, uterine pathology, delayed involution

Introduction

Uterine involution to normal position, tone, and size in the dairy cow is completed during the third or fourth week postpartum,[1] although histologic evidence indicates that endometrial repair is not complete until 42 to 50 days after parturition.[2,3] Postpartum endometritis influences uterine size and rate of involution[4] and endometritis and subsequent delayed uterine involution are associated with increased services per conception, increased calving intervals and increased culling rates.[4-7] Recent epidemiologic studies reported that postpartum endometritis decreased breeding performance,[8] stimulated PGF2α release and thus contributed to early corpus luteum (CL) regression[9] and increased days open by 20 days.[10]

Normal uterine involution is generally accompanied by massive bacterial growth. More than 93% of uteri are infected until day 15, 78% until day 30, 50% until day 45 and 9% until day 60 postpartum.[11] *Actinomyes pyogenes,* coliforms, and streptococci are the most frequent isolates.[12,13] Histologic abnormalities are primarily associated with *A. pyogenes*[13] and the development of chronic endometritis and pyometra is associated with *A. pyogenes, Fusobacterium necrophorum* and *Bacteroides melaninogenicus.*[14,15]

It is difficult to accurately diagnose endometritis by per rectal palpation in the postpartum dairy cow.[4] Clinicians at Colorado State University have identified a condition in many postpartum cows in which the tubular structure of the uterine horns is collapsible and the inner walls of the uterine horns are readily distinguishable by palpation (see Fig. 1). The uterus in these cows has returned to normal size and fluid accumulation in the lumen is not discernible. We have described this condition as a palpable uterine lumen (PUL). During the period from January 1, 1991, through September 11, 1992, 1860 calvings were recorded at one dairy near Colorado State University. At postpartum examination 179 (9.62%) of these cows were diagnosed with PUL. Evaluation of reproductive data on another dairy near the University revealed that 450 calvings occurred during the 12 month period from January 1, 1992, to December 30, 1992. Postpartum uterine examination identified 13 (2.88%) cows with a PUL. Mean days open for normal cows (122 d.) was considerably shorter than for cows with PUL (139 d.). In addition, 2 of the 13 cows with PUL were culled as open cows following multiple breedings. We suspect that uterine involution has been delayed and that a subclinical endometritis may exist in these cows which could impair future reproductive performance. We also suspect an increased incidence of *A. pyogenes* and/or other gram negative anaerobes.

The postpartum uterine condition, PUL, has not been reported in the literature; however, knowledge of the physiologic condition of uteri that have PUL may be clinically important. If PUL is associated with pathologic change, it may be a valuable diagnostic tool to the veterinarian in detecting delayed postpartum involution and subclinical infection, as well as selection of appro-

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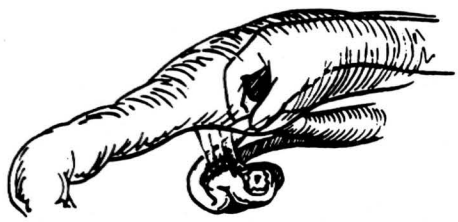
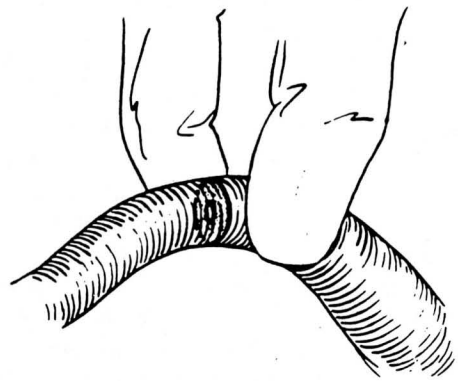
priate therapy. Our objective was to compare the condition of uteri in cows determination to have a PUL at their first postpartum examination with the condition of uteri in cows with normal reproductive tracts at first postpartum examination.

Figure 1. PUL visualization.

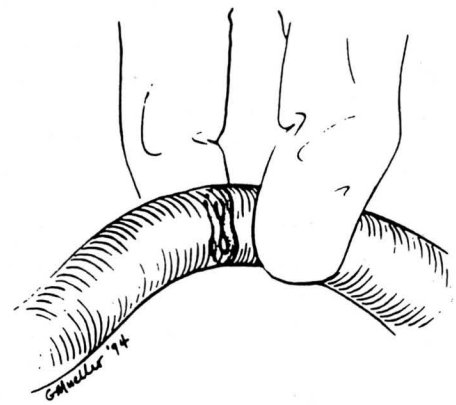
A. Per rectal palpation of the bovine uterus.



B. Normal postpartum uterus.



C. Palpable uterine lumen (PUL): note the collapsible tubular structure.



Materials and Methods

Routine uterine examination via per rectal palpation was conducted at biweekly intervals on all lactating cows between 28 and 42 days post partum at a large (1500 cow) commercial dairy near the Veterinary Teaching Hospital at Colorado State University. Fifteen cows with PUL were identified and each cow with a PUL was matched for parity and stage of lactation with a cow with a normal reproductive tract at the same herd visit. Identification of all cows to be enrolled was done by one clinician (third author), during biweekly herd visits. A second clinician (first author) confirmed the diagnosis via per rectal palpation on the same day but before any diagnostic procedures were initiated.

All cows (n = 30) were confined in a chute and headgate. Caudal epidural anesthesia was induced with 6 cc of 2% lidocaine utilizing a 12 cc sterile, disposable syringe and an 18-gauge, 1½ inch sterile, disposable needle. The tail was tied to prevent contamination, feces were evacuated from the rectum, and the perineal region was scrubbed with Nolvasan solution. The following diagnostic procedures were performed on each cow:

1. Two double-guarded culture swab[a] were passed into the uterine lumen via per rectal cervical manipulation and uterine contents were sampled. One swab was immediately inserted into Port-A-Cul[b] media to prevent oxygen exposure, and swabs were submitted to the Diagnostic Laboratory[c] for aerobic and anaerobic bacterial culture.
2. A uterine infusion pipette was passed into the uterine lumen via per rectal cervical manipulation and 50 cc of lactated Ringer's solution was infused into the uterus. Fluid contents were withdrawn and a sample of the contents was submitted to Clinical Pathology[d] for cytologic analysis.
3. A sterile uterine biopsy instrument adapted for bovine uterine biopsy[16] was passed into the uterine lumen and an endometrial biopsy specimen taken. These tissue specimens were placed in buffered 10% formalin solution, coded to prevent biased evaluation, and submitted to the Diagnostic Laboratory for microscopic evaluation. All tissue specimens were evaluated by the same pathologist and scored according to a modified grading system based on a mare endometrial biopsy scoring system.[17,18] Grade 1A was considered normal. Grade 1B uteri had mild endometrial changes characterized by minimal inflammation and mild glandular fibrosis. Grades 2A and 2B were assigned to uteri with moderate to severe endometritis with-

[a]Sterile McCullough Swab, McCullough Cartwright Pharmaceutical Corporation, 28147 W. Commercial Avenue, Barrington, IL 60010.

[b]Port-A-Cul, Benton Dickinson and Co., 250 Schilling Circle, Cockeysville, MD 21030.

[c]Diagnostic Laboratory, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523.

[d]Clinical Pathology, Veterinary Teaching Hospital, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523.

out or with mild glandular fibrosis, respectively. Grades 3A and 3B were assigned to uteri with moderate or severe fibrosis, respectively.

4. Whole blood and serum were collected from each cow for a complete blood count (CBC) and a routine large animal diagnostic panel (LADP) (see Table 1).

Data analysis

The outcome of interest was the association between a PUL and any pathologic changes. The Student's t-test was used to compare CBC, bacterial, cytologic and histopathologic findings between the two groups. The level of significance was $P \leq 0.10$.

Results

Results of the LADP revealed minimal variation from normal values (Table 1). Examination of LADP results indicated that variations from normal were evenly distributed between the groups and no pattern of group differences could be found. Results of CBC are reported in Table 2. Total WBC ranged from 5,100 to 18,800 cells/μl and did not vary between the groups, while fibrinogen levels ranged from 100 to 700 gm/dl and no difference was found between groups. These results and visual examination of all cows at the time of enrollment indicated that all cows were systemically healthy at the time of the study.

Analysis of uterine flush fluid is reported in Table 2. Red blood cells and epithelial cells were common in all samples of uterine flush fluid. Total WBC in uterine flush fluid ranged from 0 to 56,000 cells/μl but did not differ between the groups. Based on the proportion of neutrophils, clinical pathologist identified 6 cows (5 PUL, 1 normal) as having evidence of severe or chronic inflammation; however, differentials were not reported. Total protein levels exceeded 0.1 gm/dl in 3 cows (2 normal, 1 PUL), and specific gravity ranged from 1.004 to 1.014 and did not vary between the two groups.

Aerobic and/or facultative anaerobic bacteria were isolated from 14 of 15 cows with PUL and 13 of 15 normal cows, and anaerobic bacteria were isolated from 1 PUL cow (Table 3). Nonhemolytic streptococci, coliforms and *A. pyogenes* were the most common isolates; however, *H. somnus, M. bovis* and *Pasteurella* spp. were also recovered from several cows. The incidence of uterine infection did not differ between the groups and the various organisms isolated were evenly distributed between the two groups. Incidence of *A. pyogenes* did not differ between the groups.

Table 1. Large animal diagnostic panel (LADP).

Test	Normal range	Units
BUN	7-20	mg/dl
Glucose	55-95	mg/dl
Creatinine	0.9-1.7	mg/dl
Calcium	7.6-10.2	mg/dl
Phosphorus	4.0-8.6	mg/dl
Total protein	6.4-9.5	gm/dl
Albumin	2.5-4.3	gm/dl
Globulin	2.6-6.5	gm/dl
A/G ratio	0.5-1.3	ratio
Total bilirubin	0.1-0.4	mg/dl
CPK	57-280	IU/L
AST	40-130	IU/L
Sodium	136-147	mEq/L
Potassium	4.0-5.0	mEq/L
Chloride	95-105	mEq/L
Total CO_2	21-27	mEq/L
Anion gap	14-26	Calc

Table 2. Summary of analysis of blood and uterine flush fluid for normal and PUL cows.

		Blood samples		Uterine flush fluid		
Cow #	Group #	WBC (cells/μl)	Fibrinogen (gm/dl)	WBC (cells/μl)	Protein (gm/dl)	Sp. gr.
4743	1	10,500	300	100	< 0.1	1.006
4755	2	14,000	300	1,250	0.1	1.007
1435	1	7,500	300	1,900	1.5	1.014
570	2	12,500	200	1,000	0.1	1.007
5095	1	8,900	200	1,500	< 0.1	1.006
5086	2	10,800	300	700	< 0.1	1.006
1972	1	5,100	100	4,400	--	--
6035	2	7,200	300	300	< 0.1	1.006
5089	1	10,100	500	9,800	0.3	1.008
5036	2	10,300	400	550	< 0.1	1.005
5111	1	9,400	700	1,300	< 0.1	1.006
5112	2	14,400	500	2,500	0.1	1.007
4776	1	13,600	400	11,700	< 0.1	1.006
4785	2	15,700	400	0	< 0.1	1.006
110	1	7,400	400	35,600	< 0.1	1.006
133	2	17,300	400	56,400	0.6	1.010
9031	1	18,800	200	1,000	< 0.1	1.005
9056	2	10,700	400	400	< 0.1	1.004
722	1	8,700	300	100	< 0.1	1.004
146	2	10,800	500	600	< 0.1	1.005
1237	1	8,300	100	500	0.1	1.007
1216	2	8,100	200	10	< 0.1	1.004
1967	1	6,500	100	400	< 0.1	1.004
1338	2	8,400	100	1,500	< 0.1	1.004
545	1	--	--	300	< 0.1	1.004
1387	2	8,600	400	200	< 0.1	1.004
456	1	7,000	500	1,400	< 0.1	1.004
6282	2	10,400	300	1,400	< 0.1	1.004
412	1	12,800	400	0	< 0.1	1.005
466	2	9,600	300	3,300	< 0.1	1.005

Group 1 - normal cows
Group 2 - PUL cows

Table 3. Aerobic and anaerobic culture results from PUL and normal cows.

	Number of cows	
Bacterial Isolates	Group 1 (PUL)	Group 2 (normal)
Aerobic/Facultative		
Streptococcus spp.	3	6
E coli	6	3
A. pyogenes	3	4
H. somnus	2	3
M. bovis	2	2
Pasteurella spp.	2	1
Staphylococcus spp.	--	1
E. agglomerans	1	--
Bacillus	--	1
Anaerobic		
F. necrophorum	1	--
B. melaninogenicus	1	--

Mean uterine scores assigned to cows after microscopic evaluation were higher in cows with a PUL (Table 4). Scores ≤ 1B were assigned to 11 of 15 normal cows but only 6 of 15 PUL cows. Only 1 of 15 normal, versus 5 of 15 PUL, cows graded ≤ 2B.

Table 4. Uterine scores assigned to PUL and normal cows following microscopic evaluation.

Score	Uterine scores[a] in each group	
	Group 1 (PUL)	Group 2 (normal)
1A	1	3
1B	5	8
2A	4	3
2B	4	1
3A	--	--
3B	1	0

[a] $P < 0.10$

Discussion

Analysis of whole blood and serum samples indicated that all cows enrolled in the study were systemically healthy and unaffected by uterine inflammatory responses. No association between PUL and blood or serum parameters was found. We did not expect to find sick cows since cows enrolled in the study were in production at the time. Our data confirmed those expectations.

Evaluation of uterine flush samples indicated that cows with PUL were more likely to have evidence of inflammatory changes and the presence of neutrophils in the lumen of the uterus. However, total WBC counts, specific gravity, and total protein levels in the flushes were not associated with a diagnosis of PUL. If a diagnosis of PUL is evidence of subclinical infection, then more evidence of an inflammatory response in uterine flush fluid should be seen. However, our culture results show that bacteria were present in most uteri and did not favor uteri with PUL. Therefore, it is reasonable that similar cytologic results were found in the fluid. The increased proportion of neutrophils may be related to the pathologic changes seen during microscopic examination of biopsy specimens.

Diagnosis of PUL was not associated with an increased incidence of bacterial infection or the presence of *A. pyogenes*. Uterine culture results revealed a high incidence of bacterial infection in all cows. Because these cows had recently calved, these results were expected and are in agreement with previous reports.[11] *Streptococci* and *E coli* are common isolates from early postpartum cows that are spontaneously eliminated by the uterine defense mechanism.[12] *A. pyogenes*, *F. necrophorum* and *B. melaninogenicus* have been associated with chronic endometritis and histologic abnormalities;[13-15] however, no correlation was found between the presence of these bacterial and inflammatory changes seen during evaluation of the uterine flushes or microscopic examination of uterine tissue.

Higher uterine biopsy scores in cows with PUL indicate that more severe inflammatory responses were present. Of the 6 cows identified by clinical pathologists to have evidence of severe or chronic inflammation, 5 cows were diagnosed with PUL. All of these cows received uterine scores of 2A or greater. More research in this area is needed. Increased fibrosis of uterine glands and elevated numbers of inflammatory cells are associated with reduced reproductive efficiency in horses.[20] Although an association between increased inflammatory changes and reduced reproductive efficiency in cattle is not reported in the literature, it is possible that such an association exists. These data indicate that diagnosis of PUL is more likely to identify cows with severe inflammatory changes.

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References

1. Casida, L.E. and Vengke, W.G. Observations on reproductive processes in dairy cattle and their relation to breeding efficiency. *29th Annu Meeting Proc Amer Soc Amin Prod* 221-223 (1936). 2. Buch, N.C., Tyler, W.J. and Casida, L.E. Postpartum estrus and involution of the uterus in an experimental herd of Holsteins. *J Dairy Sci* 38:73-79 (1955). 3. Marion, G.B. and Gier, H.T. Histological and cytological changes in the bovine uterine epithelium. *J Anim Sci* 18:1552-1553 abstr (1959). 4. Tennant, B. and Peddicord, R.G. The influence of delayed uterine involution and endometritis of bovine fertility. *Cornell Vet* 58:185-192 (1968). 5. Morrow, D.A., Roberts, S.G. and McEntee, K. Postpartum ovarian activity and uterine involution in dairy cattle. *JAVMA* 12:1596-1609 (1966). 6. Sandals, W.C.D., R.A., Cote, J.F. and Martin, S.W. The effect of retained placenta and metritis complex on reproductive performance in dairy cattle - a case control study. *Can Vet J* 20:131-135 (1979). 7. Miller, H.V., Kimsey, P.B., Kendrick, J.W., Darien, B. and Doering, L. Endometritis of dairy cattle: diagnosis, treatment, and fertility. *The Bov Pract* 15:13-23 (1980). 8. Erb, H.N., Smith, R.D., Detenscu, P.A., Guard, C.L., Hillman, R.B., Powers, P.A., Smith, M.C. and White, M.E. Path model of reproductive disorders and performance, milk fever, mastitis, milk yield, and culling in Holstein cows. *J Dairy Sci* 68:3337-3349 (1985). 9. Peter, A.T. and Bosu, W.T.K. Effects of intrauterine infections on the function of the corpora lutea formed after first postpartum ovulations in dairy cows. *Theriogenology* 27:593-609 (1987). 10. Oltenacu, P.A., Frick, A. and Lindhe, B. Epidemiological study of several clinical diseases, reproductive performance and culling in primiparous Swedish cattle. *Prev Vet Med* 9:59-74 (1990). 11. Elliot, K., McMahan, K.J., Gier, H.T. and Marion, G.B. Uterus of the cow after parturition: bacterial content. *AJVR* 29:77-81 (1968). 12. Hartigan, P.J., Griffin, J.F.T. and Nunn, W.R. Some observations on *C. pyogenes* infection of the bovine uterus. *Theriogenology* 1:153-167 (1974). 13. Studer, E., Morrow, D.A. Postpartum evaluation of bovine reproductive potential: comparison of findings from genital tract examination per rectum, uterine culture, and endometrial biopsy. *JAVMA* 172:489-494 (1978). 14. Olson, J.D., Ball, L., Mortimer, R.G., Farin, P.W., Adney, W.S. and Huffman, E.M. Aspects of bacteriology and endocrinology of cows with pyometra and retained fetal membranes. *AJVR* 45:2251-2255 (1984). 15. Farin, P.W., Ball, L., Olson, J.D., Mortimer, R.G., Jones, R.L., Abney, W.S. and McChesney, A.E. Effect of *Actinomyces pyogenes* and gram-negative anaerobic bacteria on the development of bovine pyometra. *Theriogenology* 31:979-989 (1989). 16. McQueen, R.D. A biopsy instrument for obtaining bovine endometrial samples for histologic and

metabolic investigations. *AJVR* 28:1881-1885 (1967). 17. Powers, B.E., Johnson, L.W., Linton, L.B. Garry, F. and Smith, J. Endometrial biopsy technique and uterine pathologic findings in llamas. *JAVMA* 9:1157-1162 (1990). 18. Shideler, R.K., McChesney, A.E., Morrow, G.L., Voss, J.L. and Nash, J.G. Endometrial biopsy in the mare. In: *Proc Am Assoc Equine Pract* 97-103 (1977). 19. Shott, S. Statistics for Health Professionals. W.B. Saunders Company, Philadelphia (1990). 20. Van Camp, S.D. Breeding soundness examination of the mare and common abnormalities encountered. In: Current Therapy in Theriogenology. D.A. Morrow (ed), W B Saunders Company, Philadelphia 654-661 (1986). 21. Seguin. B.E., Morrow, D.A., Louis, T.M. Luteolysis, luteotasis, and the effect of prostaglandins F2α in cows after endometrial irritation. *Am J Vet Res* 35:57-61 (1974). 22. Seguin, B.E., Morrow, D.A., Oxender, W.D. Intrauterine therapy in the cow. *JAVMA* 164:609-612 (1974).

Abstract

Management aspects of induced twinning if beef suckler cows using *in vitro* fertilized embryos

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Twin and single pregnancies were induced in two groups of oestrus-synchronised beef cows by using a combination of artificial insemination and the transfer of *in vitro* fertilized (IVF) embryos. Single IVF embryos transferred non-surgically to the uterine horn contralateral to the corpus luteum of 43 previously inseminated cows resulted in a calving rate of 72 percent with a twinning rate of 38.7 percent. In 45 cows, two IVF embryos were transferred non-surgically to one uterine horn resulting in a calving rate of 51.1 percent with a twinning rate of 39.1 percent. The median gestation length for cows bearing twins was 10 days shorter than that of cows bearing single calves ($P<0.001$). The proportions of cows that received assistance at calving were similar for twin and single births (57 percent vs 45 percent, $P>0.05$), but the incidence of retained fetal membranes was much higher after the birth of twins (62 percent vs 3 percent, $P>0.001$). Nineteen percent of twin calves were stillborn compared with 6 percent of single calves ($P>0.5$). The median birthweight of the twin calves was 32 kg (68 percent of the median weight of single calves). The nutrition of twin bearing cows in late pregnancy was adequate when assessed in terms of their plasma glucose, nonesterified fatty acid and β-hydroxybutyrate concentrations. Serum immunoglobulin concentrations were similar in single and twin calves suggesting that the passive transfer of antibody was not compromised in the twin calves.