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EFFECT OF RETAINED PLACENTA ON SUBSEQUENT BACTERIOLOGICAL AND CYTOLOGICAL INTRAUTERINE ENVIRONMENT AND REPRODUCTION IN HOLSTEIN DAIRY COWS

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ABSTRACT

To determine the effect of retained placenta on the characteristics of the intrauterine environment in dairy cows, bacteriological and cytological tests were performed on intrauterine perfusion fluid. The rate of cows with more than 70% neutrophils or fewer than 40% lymphocytes in inflammatory cells was 48.0% (12/25), while the rate and with more than 50 bacterial colonies/0.1 ml of perfusate was 96.0% (24/25) at 30 d after parturition. Actinomyces pyogenes was isolated from 56.0% (14/25). At 60 d after parturition, however, these values were significantly improved to 20.0% (5/25), 48.0% (12/25) and 12.0% (3/25), respectively. No significant differences in subsequent reproductive performance were observed between cows with and without retained placenta. The results suggest that injury to the intrauterine environment caused by retained placenta is largely healed by 60 d after parturition.

Key words: cow, retained placenta, intrauterine environment, uterine perfusion , reproductive performance

INTRODUCTION

Dystocia, retained placenta, injury to the birth canal and uterine prolapse are considered responsible for delaying the cleansing of the uterus following parturition. Of these factors, retained placenta occurs most frequently and can seriously affect subsequent fertility by causing metritis (1,10,15). Adequate treatment for placental retention is, therefore, considered important for the management of reproduction.

Knowledge of characteristics of the intrauterine environment following placental retention is needed to establish effective measures

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for improving the reproductive efficiency in cows with retained placenta (9,14). Kaneko et al. (11) examined parameters of bacteriological and cytological uterine perfusates from apparently healthy cows on the day following artificial insemination, and proposed objective criteria for defining deterioration. The purpose of the present study was to determine the characteristics of the intrauterine environment in cows with retained placenta by bacteriological and cytological examinations of intrauterine perfusion fluid and to evaluate the effect on subsequent fertility.

MATERIALS AND METHODS

Cows and Fluid Collection

This study was conducted between October 1992 and September 1993, using 100 Holstein cows housed in barn stalls at 8 dairy farms. The average milk production at each farm was 7500 to 9500 kg /head /305 d. Fifty cows had not expelled the placenta within 24 h post calving and were assigned to the experimental group. The remaining 50 had expelled the placenta and thus were pair-matched by age, season of parturition and farm to the experimental group. No treatment was administered to cows with retained placenta, and they spontaneously expelled the placenta within 7 to 10 d. Cows with retained placenta were allocated alternately to Groups RP30 and RP60. The paired control cows were allocated to Groups C30 and C60.

The intrauterine perfusion fluid was collected by the method of Kaneko et al. (11) from Groups RP30 and C30 at 30 d after parturition, and from Groups RP60 and C60 at 60 d after parturition. A vaginal speculum was inserted into the vagina after cleansing of the vulva with a disinfectant, and the tip of a balloon catheter (Terumo Inc., Tokyo, Japan, Fr 22) was inserted into the cervix as deep as possible without touching the vaginal wall. Then the vaginal speculum was removed, the balloon catheter was advanced into the uterus using the recto-vaginal method, and the balloon was inflated. Sterile physiological saline (100 ml) was infused into the uterus through a balloon catheter and recovered by gently massaging the uterus .

Bacteriological Examination of Intrauterine Perfusion Fluid

The perfusion fluid (10 ml) was centrifuged at 1,000 x g for 10 min, and, after removal of the supernatant, the sediment was resuspended in 1 ml of physiological saline . An aliquot of the resuspended sediment(100μl) was applied to soy agar with 5% sheep blood, and incubated aerobically at 37℃ for 48 h. Using the criteria of Kaneko et al. (11), samples showing

growth of more than 50 identical colonies were defined as positive for bacteria, and were considered to indicate bacteriological deterioration of the intrauterine environment. Gram-negative, atypical, pine leaf-like rods, which showed hemolytic reaction on sheep blood-containing agar medium and were negative to the catalase test, were judged to be Actinomyces pyogenes. Samples showing the growth of more than one A. pyogenes colony were defined as positive for A. pyogenes.

Cytological Examination of Intrauterine Perfusion Fluid

The perfusion fluid (10 ml) was centrifuged as described above and the sediment was smeared on a glass slide, dried in air, fixed for 3 min with methyl alcohol and then sediment was stained with Giemsa. A total of 200 cells was counted at ×1,000 in each specimen and classified into neutrophils, eosinophils, basophils, lymphocytes and macrophage-like cells. The percentages of neutrophils and lymphocytes were calculated and recorded. Using the criteria of Kaneko et al. (11), specimens with a neutrophil ratio exceeding 70% or a lymphocyte ratio below 40.0% were considered to indicate a cytologically poor intrauterine environment.

Investigation of Reproductive Performance

Cows were examined for reproductive performance after parturition (n=17, 20, 20 and 19 from Groups RP30, RP60, C30 and C60, respectively). The number of days from parturition to initial insemination, the number of days until conception and the number of inseminations required to achieve conception in these cows were determined.

Statistical Analysis

The positive rates for bacteria and for A. pyogenes were compared between groups by use of the Chi-square test. The neutrophil ratio and the lymphocyte ratio in sedimented inflammatory cells, and the reproductive performances were compared by Student's *t*-test.

RESULTS

Bacteria in the Intrauterine Perfusion Fluid

The detection rate for bacteria and for A. pyogenes was the highest in cows with retained placenta collected 30 d after parturition, and it was significantly higher than in any other group ($P<0.05$ and $P<0.01$, respectively. Table 1).

Table 1. Proportion of cows with and without retained placenta showing positive cultures of aerobic bacteria and of A. pyogenes in the intrauterine perfusion fluid collected at predetermined intervals after parturition

	Cows n	Bacteria positive (%)	A. pyogenes positive (%)
Cows with retained placenta			
Group RP30[a]	25	12 (48.0)[c]	14 (56.0)[e]
Group RP60[b]	25	5 (20.0)[d]	3 (12.0)[f]
Control cows			
Group C30[a]	25	7 (28.0)	1 (4.0)[f]
Group C60[b]	25	4 (16.0)[d]	1 (4.0)[f]

[a]Examined at 30 days after parturition.
[b]Examined at 60 days after parturition.
cd,ef; P<0.05.

Cells in Intrauterine Perfusion Fluid

The mean percentage of neutrophils in Group RP30 was the highest (P<0.01) than in any other group. A significant difference (P<0.01) was also found between Group C30 and Group C60. The mean percentage of lymphocytes in Group RP30 was the lowest (P< 0.01) of all the other groups. Again, there was a significant difference (P< 0.01) between Group C30 and Group C60 (Table 3).

The number of cows with more than 70% neutrophils or fewer than 40% lymphocytes in the inflammatory cells was the highest in Group RP30 at 96.0% (24/25). It was lower (48.0% ; 12/25) in Group RP60, and a significant difference (P<0.01) was found between the 2 groups. The number of cows with more than 70% neutrophils or fewer than 40% lymphocytes in the inflammatory cells was also significantly (P<0.01) higher (68.0% ; 17/25) in Group C30 than in Group C60 (24.0% ; 65/25).

Among the bacterial species other than A. pyogenes that were isolated in each group were Streptococcus spp. (isolated most frequently), Escherichia coli, Enterococcus spp, and Enterobacter spp, which may have originated from feces (Table 2).

Table 2. Bacterial species other than A. pyogenes isolated from the intrauterine perfusion fluid of cows with and without retained placenta, collected 30 and 60 days after parturition

Group	Bacterial species	Number of cows
Group RP30[a]	Streptococcus spp.	3
	Corynebacterium spp.	2
	Enterobacter spp.	2
	Pasteurella spp.	1
Group RP60[b]	Streptococcus spp.	1
	Staphylococcus spp.	2
	Pasteurella spp.	1
Group C30[c]	Streptococcus spp.	2
	Escherichia coli	4
	Bacillus spp.	2
Group C60[d]	Streptococcus spp.	2
	Enterococcus spp.	2

[a] Cows with retained placenta collected 30 d after parturition.
[b] Cows with retained placenta collected 60 d after parturition.
[c] Cows without retained placenta collected 30 d after parturition.
[d] Cows without retained placenta collected 60 d after parturition.

Table 3. Percentage of neutrophils and lymphocytes (mean±SD) in the intrauterine perfusion fluid of cows with and without retained placenta, collected 30 and 60 days after parturition

	Cows n	Percentage of neutrophils (%)	Percentage of lymphocytes (%)
Cows with retained placenta			
Group RP30	25	83.5±17.7[a]	14.4±16.0[e]
Group RP60	25	52.4±29.5[b]	41.7±27.4[f]
Control cows			
Group C30	25	63.9±26.0[c]	31.0±23.3[g]
Group C60	25	36.9±26.3[d]	55.4±27.1[h]

ab,ac,ad,cd,ef,eg,eh,gh; P<0.01.

Reproductive Performance of Cows

There were no significant differences among or between the Groups of cows with retained placenta (Groups RP30 and RP60) and the control cows (Groups C30 and C60) in the number of days from parturition to initial insemination (90.0±28.5, 83.0±32.2), the number of days to

conception (124.7 ± 56.4, 131.2 ± 66.0) and the number of artificial inseminations (1.78±1.03, 1.97±1.06,) required for conception.

DISCUSSION

Kaneko et al. (11) examined parameters of bacteriological and cytological uterine perfusates from 217 Holstein cows on the day following artificial insemination. They found that in the cows showing more than 50 bacterial colonies/0.1 ml of perfusate the conception rate (5.6%) was significantly lower. Cows with more than 70% neutrophils and those with fewer than 40% lymphocytes in inflammatory cells also showed reduced conception rates of 27.6 and 37.9%, respectively. In the present study, we used those criteria to evaluate the intrauterine environment.

The rate of bacterial detection in the intrauterine perfusion fluid from the cows with retained placenta was high (48.0%) at 30 d after parturition. Moreover, A. pyogenes was isolated from 56.0% of the cows. In the control cows, the rate of bacterial detection at 30 d after parturition was also high (28.0%), but A. pyogenes was isolated from only 4% of the animals. This indicates that the uterus of cows with retained placenta is prone to infection with A. pyogenes, a finding that is substantially in accord with the report of Olson et al. (12). A. pyogenes induces metritis by synergism with gram-negative bacilli such as Fusobacterium necrophorum or Dichelobacter melaninogenicus, and this infection reduces fertility (13). The rate of bacterial detection and rate of A. pyogenes isolation were both decreased at 60 days after parturition, and were no longer significantly different from those of the control cows. Escherichia coli, Enterococcus spp, and Enterobacter spp. were also isolated. Although this finding might suggest fecal contamination during the sampling procedure, the number of cases was not large.

In the cytological examination, the percentage of neutrophils was significantly higher and the percentage of lymphocytes significantly lower at 30 d after parturition in the cows with retained placenta than in the control cows. The number of cows whose intrauterine environment was judged as cytologically poor was as high as 96.0%. These values became normalized at 60 d after parturition, and were no longer significantly different from those of the control cows. It appears that retained placenta does adversely affect the postpartum intrauterine environment, but the injury is repaired by 60 d after parturition.

Retained placenta has been reported to induce metritis and thereby to reduce subsequent fertility (1-8, 15). In this study, no significant

difference in reproductive performance was found between the cows with retained placenta and the control cows. It has been reported that fertility was not affected in cows with retained placenta if metritis was not induced after placental retention or if the cows had recovered from metritis by the time of insemination (2,15,16). It is concluded that in most cows particular treatment for retained placenta is not needed.

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