The Effect of PGF2α, GnRH, E2 or Antibiotics on the Intrauterine Environment and Reproduction in Holstein Dairy Cows with Retained Placentas

Kazuyuki Kaneko*
Department of Veterinary Obstetrics and Gynecology, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 229-8501, Japan

Abstract

PGF2α, GnRH, estradiol (E2), or antibiotics were given to dairy cows with retained placenta after parturition to establish the most suitable treatment to prevent the development of endometritis. One hundred and seventy five parous Holstein cows with retained placenta were allocated to six treatment groups, and groups were given PGF2α, GnRH, E2, or antibiotics at 30 or 45 days after parturition. The intrauterine perfusion fluid was collected at 60 days after parturition, and a bacteriological and cytological examination was conducted. Reproductive performance was also investigated. The detection rate for Trueperella pyogenes in the intrauterine perfusion fluid in each group ranged from 0% to 16%, and T. pyogenes was not isolated in cows that were given PGF2α or E2. The percentage of neutrophils observed in the intrauterine perfusion fluid ranged from 37.8 ± 5.3 to 56.3 ± 4.7, and it was lowest in cows given PGF2α and highest in cows given GnRH. The mean number of days from parturition to initial insemination, the mean number of days from parturition to conception and the mean number of artificial inseminations required for conception ranged from 85.9 ± 5.5 to 102.7 ± 6.0, 103.8 ± 9.0 to 162.3 ± 18.6 and 1.5 ± 0.2 to 2.6 ± 0.3, respectively. All three parameters were best in cows that were given a combination of PGF2α and E2. The results suggest that administration of PGF2α or E2, or ideally both, might be a suitable treatment for cows with retained placenta. However, treatment with GnRH might delay the cleansing of the uterus.

Keywords: Cattle; Endometritis; Reproductive performance; Retained placenta; Uterine perfusion

Introduction

Dystocia, retained placenta, injury of the birth canal and uterine prolapse in Holstein dairy cows are considered to be responsible for the delay in purification of the uterus following parturition. Of these factors, retained placenta is the most frequent and seriously affects subsequent fertility owing to the development of metritis [1-4]. Proper treatment for placental retention is, therefore, considered important in the management of reproduction. There are many reported treatment methods to prevent endometritis after retained placenta, and the application of PGF2α, GnRH, estradiol (E2), or antibiotics is preferentially used [1,5-7]. Kaneko et al. [8,9] reported that the uterus of cows with retained placenta was easily infected with Trueperella pyogenes (T. pyogenes), and that they tended to suffer from endometritis. Furthermore, the percentage of neutrophils in the cells observed in the intrauterine perfusion fluid was high in the uterus after the placenta was retained when compared to a uterus without retained placenta. The purpose of this study was to examine the effects of administration of PGF2α, GnRH, E2 or antibiotics on the presence of T. pyogenes in the uterus, the percentage of neutrophils, and the subsequent reproductive performance in Holstein dairy cows with retained placenta.

Materials and Methods

Animals

Two hundred parous Holstein cows ≥ 2 years old (3.8 ± 0.5: mean ± SEM) were used. The cows were housed in barn stalls at 15 dairy farms, fed a total mixed ration and had free access to water. Their BCS was 2.5 to 4.0, and their milk volume was 25 to 40 Kg/day. Of the 200 cows, 175 did not expel the placenta within 24 hours of calving and were diagnosed with retained placenta. The remaining 25 cows expelled their placenta within 24 hours of calving and were assigned to the control group (Group C). All cows diagnosed with retained placenta spontaneously expelled the placenta within 7-10 days of parturition without any medical intervention, and they were allocated into one of six treatment groups. All treatments were carried out with approval from the ethics committee of Azabu University.

Treatment

Cows with retained placenta were randomly assigned into one untreated group and the following six treatment groups each consisting of 25 cows. Group NT: no treatment was given. Group GnRH: cows were treated with an IM injection of 200 μg of fertirelin acetate at 30 days' post parturition. Group ABP: cows were treated with an IU infusion of 500 mg of ampicillin administered at 30 days' post parturition. Group FS: cows were treated with an SC injection of 1 mg of fenprostalene at 30 days' post parturition. Group DP: cows were treated with an IM injection of 25 mg of dinoprost at 45 days' post parturition. Group E+DP: cows were initially treated with 5 mg of estradiol benzoate administered by IM injection at 30 days' post parturition, followed by an IM injection of 25 mg dinoprost at 45 days post parturition.

*Corresponding author: Kazuyuki K, Department of Veterinary Obstetrics and Gynecology, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 229-8501, Japan, Tel: 0428502454; Fax: 042-850-2454; E-mail: kaneko@azabu-u.ac.jp

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Collection of intrauterine perfusion fluid

The intrauterine perfusion fluid was collected using the method of Kaneko et al. [8] at 60 days after parturition. A 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 deeply as possible without touching the vaginal wall. The vaginal speculum was then removed, the balloon catheter was advanced into the uterus using the reto-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 × g for 10 min and after removing the supernatant, the sediment was resuspended in 1 ml of physiological saline. An aliquot of the resuspended sediment (100 μL) was applied to soy agar with 5% sheep blood, and incubated at 37°C for 48 hours. Gram-negative, atypical, pine leaf-like rods, which showed a hemolytic reaction on sheep blood-containing agar medium and were catalase negative, were judged to be T. pyogenes. According to Kaneko et al. [8], a sample showing growth of one or more T. pyogenes colonies was defined as positive for T. pyogenes.

Cytological examination of intrauterine perfusion fluid

The perfusion fluid (10 mL) was centrifuged as described above and the sediment was smeared onto a glass slide, air dried, fixed for 3 mins with methyl alcohol and stained with Giensa stain. Two hundred cells were counted at × 1,000 for each specimen and classified into neutrophils, eosinophils, basophils, lymphocytes and macrophage-like cells. The percentage of neutrophils was calculated.

Reproductive performance

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 investigated.

Statistical analysis

The T. pyogenes detection rate was compared between groups using a chi-square test. The percentage of neutrophils and the reproductive performance were compared between groups using Student’s t test.

Results

The rate of detection of T. pyogenes

The rate of T. pyogenes detection ranged from 0% (Group E, Group FS and Group E+DP) to 16% (Group NT). However, there was no significant difference between the groups (Table 1).

The percentage of neutrophils in intrauterine perfusion fluid

The mean percentage of neutrophils ranged from 37.8 ± 5.3 (Group C) to 56.3 ± 4.7 (Group GnRH). Group DP was found to have the lowest (40.7 ± 5.9) mean percentage of neutrophils of the treated groups. The mean percentage of neutrophils in Group GnRH was significantly higher than the means in Group C, Group E, Group FS, Group DP and Group E+DP (P<0.05) (Table 1).

Reproductive performance

The number of cows in each group that was used for reproduction after parturition was 19 in Group C, 20 in Group NT, 19 in Group GnRH, 19 in Group ABP, 16 in Group E, 15 in Group FS, 19 in Group DP and 18 in Group E+DP. The mean number of days from parturition to initial insemination ranged from 85.9 ± 5.5 (Group E+DP) to 102.7 ± 8.0 (Group E). The mean number of days from parturition to conception ranged from 103.8 ± 9.0 (Group E+DP) to 162.3 ± 18.6 (Group ABP). The mean number of artificial inseminations required for conception ranged from 1.5 ± 0.2 (Group E+DP) to 2.6 ± 0.3 (Group ABP). All three parameters were lowest in Group E+DP and there was a significant difference between Group E+DP and some of the other groups in the three parameters (Table 2).

Discussion

Each treatment method used in this study was based on the following facts. E2 facilitates the evacuation of exudate in the uterus by causing contraction of the uterus and relaxation of the cervix [10]. Furthermore, E2 increases peripheral blood neutrophil counts [11,12] and its function is enhanced under estrogen domination [13]. GnRH stimulates follicular growth after parturition and increases the blood estrogen level. For this reason, GnRH has been used to treat endometritis after retained placenta [5,14]. PGF2α regresses the corpus luteum and induces estrous causing blood estrogen levels to increase. It was reported previously that PGF2α is effective for treatment of endometritis [15-17]. In particular, fenprostalene was reported to exert a strong direct contractile action on the myometrium as well as causing a luteolytic effect. Furthermore, the effect of fenprostalene is long-lasting [18,19]. The beneficial effect of antibiotics as an intrauterine therapy has been reported for the treatment of retained placenta or endometritis in dairy cows [18,20]. There was no significant difference between the groups in the detection rate of T. pyogenes. Kaneko et al. reported that T. pyogenes...
was isolated from 56% of cows with retained placenta at 30 days after parturition, but this decreased to 12% at 60 days after parturition in cows that spontaneously expelled their placenta without any treatment [9]. In this study, the rate of *T. pyogenes* detection in Group NT was 16% which was similar to Kaneko’s findings. However, *T. pyogenes* was not isolated in Group E, Group FS and Group E+DP. This is an important result because *T. pyogenes* induces metritis by synergism with gram-negative bacilli such as Fusobacterium necrophorum or Dichelobacter melaninogenicus which have been shown to reduce fertility [2,6,21,22]. 

*T. pyogenes* was isolated from two cows in Group ABP. It was reported that intrauterine infusion of antibiotics was ineffective in preventing intrauterine infection in a postpartum dairy cow with retained placenta [23,24].

Kaneko et al. performed a cytological examination of intrauterine perfusions and reported that the percentage of neutrophils was higher in the uterus of cows with retained placenta at 30 or 60 days after parturition [9]. In this study, the percentage of neutrophils was lowest in Group C indicating that the retained placenta has a negative effect on clearance of the intrauterine environment. The percentage of neutrophils was highest in Group GnRH. GnRH induces ovulation and development of the corpus luteum causing the progesterone level in the blood to increase. Progesterone causes closure of the cervix, reduction of uterine contractions and increases the susceptibility of the uterus to infection [25]. Therefore, GnRH administration at around this time, when bacterial infection, especially *T. pyogenes*, is dominant [9], might be disadvantageous to the cleansing of the uterus following retained placenta [25,26]. On the other hand, it was relatively low in the groups to which PGF2α or E2 were given [Group E, Group FS, Group DP and Group E+DP]. This might suggest that PGF2α or E2 have a positive effect on clearance of the intrauterine environment. PGF2α administration has been shown to induce estrus and positively influence uterine involution in cows with luteal tissue [27].

The reproductive performance in Group E+DP was the best in terms of the number of days from parturition to initial insemination, the number of days until conception and the number of inseminations required to achieve conception. This was even better than in cows that did not have retained placenta (Group C). The absence of any improvement in reproductive performance in Group E and Group DP indicates that there might have been a synergism between estradiol benzoate and dinoprost. Estradiol, in combination with dinoprost, has been shown to reduce intrauterine bacterial contamination [28]. In addition, only Group E+DP received two treatments, and repeated exposure of the uterus to estrogens might have facilitated the clearance of the intrauterine environment.

Based on the detection rate of *T. pyogenes*, the cytology results, and the reproductive performance, we propose that administration of PGF2α or E2, especially in combination, might be a suitable treatment for cows with retained placenta. We also provide evidence that the use of GnRH might cause a delay in the cleansing of the uterus.

Conflicts of Interest

Authors have none to declare.

References


