FERTILITY PARAMETERS OF DAIRY COWS WITH RETAINED PLACENTA OR ENDOMETRITIS TREATED WITH INTRAUTERINE POVIDONE IODINE FOAM

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Received for publication February 17, 2009

Abstract

The aim of the study was to evaluate the reproductive indices in cows with retained placenta and endometritis in response to D-cloprostenol or povidone iodine (PI) foam treatment, as well as bacterial clearance and histopathological changes after PI foam intrauterine administration. The examinations were performed on a total of 113 Brown Swiss dairy cows. In experiment I, cows with retained placenta (n=42) and endometritis (n=50) were administered randomly with either PI foam (2%) (20-150 ml, intrauterine) or PGF2α (150 µg, i.m.). The uterine microbial flora was also monitored in the cows before and after PI foam administration. In experiment II, 21 cows without reproductive diseases were administered with PI foam (20 ml, intrauterine) 10, 20, and 30 d before slaughtering to evaluate the histopathology of the uterine tissue. In the cows with retained placenta, there were no effects from the treatment on the number of days to 1st service (d), days open (d), 1st service conception rate (%), and insemination index, which averaged 100, 124, 28.6, and 2.19, respectively. In the cows with endometritis, PI foam administration decreased the number of days to 1st service and days open - 13 and 17 d - as compared to PGF2α administration, without altering the 1st service conception rate (26.4%) and insemination index (2.32). E. coli was the most common bacterium in these cows. On days 5 and 15, relative to PI foam administration, 56 and 100% microbial recovery were achieved. Furthermore, no degenerative changes in the laminal epithelium of uterus were detected upon PI foam administration. In conclusion, the microbial recovery and shortened days to 1st service and days open may suggest that a low level of PI foam can be considered as a therapeutic agent against retained placenta and endometritis.

Key words: cow, endometritis, retained placenta, povidone iodine foam, PGF2α.

Reproductive efficiency constitutes one of the indispensable elements for profitable dairy production (24) because its failure is associated with economic loss mainly due to prolonged days open, reduced conception rate, drug cost, and increased culling rate (5). Uterine diseases caused by the bacterial contamination of uterine lumen after parturition lead to interference with uterine functions (40). Bacteria are isolated from the uterine lumen of many cows until 2 weeks postpartum (38). It has been reported that there were pathogenic bacteria in 10%-17% of cows, which causes uterine inflammations, and which were detectable by physical examination. Moreover, the bacteria were reported to be eliminated spontaneously within 5 weeks postpartum in many cows (2, 23). Hussain (16) showed that the bacterial contamination rate was 93% in early postpartum, and that the rate decreased to 9% by days 40-60 postpartum. The presence of pathogenic bacteria in the uterus causes inflammation and pathomorphological lesions in the endometrium, which delays uterine involution, as well as endangering embryo survival (37). In addition to repressing the release of pituitary LH, bacterial products or inflammations compromise postpartum follicular development and ovulation mechanism in cattle (31, 38). Thus, infections extend the period of days open and days to 1st service conception, and decrease the rate of conception (2, 5, 42).

Many antimicrobial agents and hormones are used in the treatment of postpartum uterine infections (44). Though pathogenesis and the diagnosis of postpartum uterine diseases resemble each others, discussions about optimal treatment still continue. PGF2α analogues have been widely used in cows with reproductive problems during the postpartum period (18, 34). Studies indicating negative or no additional positive effects of povidone iodine (PI) administration on fertility are available (14). Despite having many negative effects, iodine preparations have also been used in cows for 50 years because of their low cost, proven treatment effect, and the scarcity of their residual period in meat and milk (4). It has also been used in humans for genital canal infections (26). The objectives of this experiment were to evaluate the reproductive indices in cows with retained placenta and endometritis in response to the
administration of D-cloprostenol, a PGF2α analogue, and PI foam (2%) as well as bacterial clearance and histopathological changes before and after PI foam administration.

**Material and Methods**

**Animals and experimental groups.** In experiment I, a total of 92 Brown Swiss dairy cows with retained placenta (n=42) and endometritis (n=50) were obtained from a local farmer between 2006 and 2008. Cows with different reproductive (ovarian cyst, etc.) and metabolic problems were not included. Herd health was monitored from parturition to conception. The cows were categorised as those with retained placenta upon diagnosis by having their foetal membranes saved manually within 2 d after parturition, and as those without retained placenta but having suffered from endometritis from days 21 to 28 postpartum as diagnosed by ultrasonography and vaginoscopy. Cows with either retained placenta or endometritis were then assigned randomly to one of two treatments: intrauterine administration of 2% PI foam (Piyofom, Galenka, Turkey) (PI group), or intramuscular administration of PGF2α (D-clorprostenol, 150 µg) (Dalmazin, Vetas, Turkey) (PG group). The dose of PI foam was 150 and 20-30 ml for cows with retained placenta and endometritis, respectively. The degree of the severity of endometritis development was classified as category 1, 2, or 3 if the rectal findings were normal and there was seromucous discharge with flakes of pus; if the rectal findings were normal or with a slight asymmetry, the cows had enlarged horns or thickened uterine wall, and there was mucopurulent discharge; if there was an enlarged uterus and there was putrid, foul smelling discharge or necrotic tissue in discharge, respectively (42) (Table 1). The milk of the cows treated with PI foam was discharged for 3 d due to public concern. In this experiment, microbial recovery upon PI foam administration was also attained.

In experiment II, 21 cows without reproductive and metabolic problems, but planned to be culled, were subjected to PGF2α and PI foam administrations in the cows with retained placenta and endometritis. None of the response variables differed in the cows with retained placenta and endometritis. None of the response variables differed in the cows with retained placenta and endometritis. None of the response variables differed in the cows with retained placenta and endometritis. None of the response variables differed in the cows with retained placenta and endometritis. None of the response variables differed in the cows with retained placenta and endometritis. None of the response variables differed in the cows with retained placenta and endometritis. None of the response variables differed in the cows with retained placenta and endometritis.

**Response variables.** The farm was visited once a week unless there was a dystocia or reproductive problem. In order to attain the reproductive parameters, all cows were observed for 30 min three times a day starting from day 42 postpartum. Cows exhibiting oestrus were artificially inseminated, and those with repeating signs of oestrus were re-inseminated; otherwise, pregnancy was checked within 35 d after insemination using ultrasonography.

**Bacterial examination.** To evaluate the bacterial clearance, intrauterine swaps were only taken from the cows administered with PI foam in experiment I before treatment of retained placenta and endometritis. The samples were aerobically incubated at 37°C using sheep blood agar (Oxoid Ltd, UK), eosin methylene blue agar (Oxoid), McConkey agar, and Sabouraud dextrose agar (Merck, Germany). Material was also seeded on anaerobic agar plates (Merck) for the isolation of anaerobic bacteria. The cultures were placed in an anaerobic jar (GasPak, Becton Dickinson, USA). The aerobic and anaerobic bacteria, and fungi were identified microscopically according to their reproductive properties (haemolysis, pigment, etc.) using Gram staining, spore forming, and various biochemical tests. The API half-automated identification system (bioMérieux, France) was used for the strains that were not identifiable by the traditional methods (13, 21). The same procedure was applied on days 5, 10, 15, and 20 post-administration on the samples taken from the corpus or cervix uteri, or the cervical mucus in the case of the closed cervix being closed.

**Histopathological examination.** After slaughtering the cows in experiment II, the uterine tissues were removed and fixed in 10% buffered formalin, and then subjected to routine tissue processing. The 5 µ thick cross sections cut from the paraffin blocks were stained with haematoxylin–eosin (HE) (33). The preparations were examined under light microscopy.

**Statistical analysis.** In experiment I, the response variables (days to 1st service, days open, and insemination index) were subjected to one-way ANOVA (41). Furthermore, microbial clearance was described by using the Proc. Means and Plot Procedures. The conception rate at 1st and 2nd service by the treatment were subjected to χ² test (36). Statistical significance was declared as P<0.05.

**Results**

The incidences and severity of endometritis in the experimental groups showed no differences (Table 1). Table 2 summarises the fertility indices in response to PGF2α and PI foam administrations in the cows with retained placenta and endometritis. None of the response variables differed in the cows with retained placenta. In the cows with endometritis, however, the administration of PI foam shortened the days to 1st service and days open by 13 and 17 d respectively, compared with injections of PGF2α (P<0.05 for both). The treatment method did not alter the conception rate at 1st and 2nd service and the insemination index in cows with endometritis.
Table 1
Incidence of endometritis at varying degree of severity

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Povidone iodine (2%)</th>
<th>PGF2α</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/11</td>
<td>5/11</td>
</tr>
<tr>
<td>2</td>
<td>17/30</td>
<td>13/30</td>
</tr>
<tr>
<td>3</td>
<td>4/9</td>
<td>5/9</td>
</tr>
</tbody>
</table>

1 Categories based on Studer and Morrow (52).

Table 2
Effects of PI (2%) and PGF2α on mean fertility parameters in cows with postpartum uterine problems

<table>
<thead>
<tr>
<th>Groups</th>
<th>Response variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days to 1st service (day)</td>
</tr>
<tr>
<td>Retained placenta</td>
<td></td>
</tr>
<tr>
<td>PI, n=23</td>
<td>96.52 ±4.63</td>
</tr>
<tr>
<td>PGF2α, n=19</td>
<td>104.74 ±4.07</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
</tr>
<tr>
<td>Endometritis</td>
<td></td>
</tr>
<tr>
<td>PI, n=27</td>
<td>104.11 ±3.69</td>
</tr>
<tr>
<td>PGF2α, n=23</td>
<td>117.17 ±3.09</td>
</tr>
<tr>
<td>P value</td>
<td>*</td>
</tr>
</tbody>
</table>

1 CR - conception rate; NS - not significant; ± SE; * P<0.05.

Fig. 1. Microbial isolate prior to intrauterine PI foam (2%) application (A) and microbiological recovery or clearance rate after the treatment (B).
The uterine microbial flora prior to PI foam administration is depicted in Fig. 1A. Most infections were caused by “mix” agents (30%), predominantly by *E. coli*. Specifically, *E. coli* was the most predominant bacteria causing uterine infection (22%), which was followed by *Arcanobacterium pyogenes* (12%), non-haemolytic Streptococcus (10%), *S. aureus* (8%), β-haemolytic *Streptococcus* (6%), *Candida albicans* (6%), and *P. aeruginosa* (6%). Microbial recovery or clearance reached about 50% on day 5 and 100% on day 15 following PI foam administration, relative to the bacteria enumerated prior to its administration (Fig. 1B).

The histopathological examination revealed no degenerative changes in the epithelium of the uteri 10, 20, and 30 d after PI foam administration (experiment II). However, there were subepithelial mononuclear cell infiltrations starting from day 10 post administration of the PI foam. There was also a connective tissue cell infiltration starting from day 10 post administration of the PI foam, relative to the bacteria enumerated prior to its administration (Fig. 1B).

In the later experimental days, there were inflammatory reactions in the uterus, which were highly varied.

**Discussion**

The uterus is routinely contaminated by bacteria during the early postpartum period. The uterine defence mechanism reacts against the invasion by environmental microorganisms into the genital canal by, anatomically, simple or pseudo stratified columnar epithelium covering the endometrium, chemically, by mucus secretion released from the endometrial glands, immunologically, by polymorph nuclear inflammatory cells, and by humoral antibodies (6). However, a decrease in the ability of cows with various periparturient problems to control uterine infections has been observed. Besides, the *lochia* is a suitable environment for bacterial invasion to contribute to the development of an infection (30). The primary objective of the reproductive management is to allow the cows to become pregnant within an optimal duration, and to limit the period between two calvings. However, the occurrence of reproductive diseases interferes with this objective (40) through prolonging days open and days to 1st service, and increasing the insemination index (2, 28). Thus, the efficacy of treatment, in terms of time, and the cost effectiveness of the application, is of great importance. The administration of PGF2α is very common in a variety of reproductive diseases, and to encourage muscle contraction in the cases of retained placenta and endometritis. However, it has an indirect bactericidal effect. Povidone iodine is well known for its antiseptic properties. However, high doses of the compound (>10%) may exert a cytotoxic effect on the maternal tissues while killing bacteria. Thus, in this experiment, a low dose (2%) of PI in the form of foam was employed.

The removal of the foetal membranes manually and the local drug administrations are still being discussed (1, 8, 32). Several studies have shown that intrauterine manipulations weaken the uterine defence mechanism (32) and deteriorate the fertility parameters (1). However, a retrospective study involving 1,702 cows with retained placenta reported that the attempts at manual removing had some positive effects on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10). The radical treatment of foetal membranes in conjunction with local or systemic antibiotic administration has also been suggested (9). To benefit from its antibacterial property, we employed PI foam instead of an antibiotic that has a residual effect on the reproductive performance (10).
Microbial clearance and the possible effect on CL lysis through superficial necrosis in the endometrium, and the regeneration ability thereafter (4), could support the efficacy of PI in the cases of endometritis.

There is no direct relationship between the hygiene of the environment in which the cows live and the uterine bacterial flora. Uterine infections are closely related to attempts to help parturition and retained placenta cases in some instances (29). In this study, due to the young parity of the cows, assisted parturition through possible contamination could have contributed to the yielding of a significant number of cows with retained placenta and endometritis (19). Similar to the study by Sheldon et al. (38), *E. coli* was the most predominant cause of uterine diseases in this experiment.

The duration of the infection and, consequently, the efficacy of the treatment can contribute to the recovery from reproductive disorders (25). In agreement with the microbial recovery found in this study, other studies also stressed the bactericidal effect of PI. Vorherr et al. (45) reported that PI administration killed 99% of bacteria on the vaginal wall and that its effectiveness was not influenced by serosanguineous, mucoid or white-yellowish vaginal discharge. Munari et al. (27) reported an 88.9% microbiological recovery in the uterus of cows 7 d after the administration of a single-dose of PI solution. Monif et al. (26) reported that aerobic and anaerobic bacteria fell to basal levels within 30-120 min. In that study, the rapid effect could be related to the tested dose (10%). Bacteria clearance was halved on day 5 after PI foam administration relative to that pre-administration, which was totally eliminated on day 15 post-administration of PI foam, suggesting the existence of a bactericidal effect of PI foam at 2%.

Iodine causes an abrasion of the surface of the epithelium, and leads to the occurrence of inflammatory reactions within a day in cows (12) and mares (3). The inflammatory reactions lessened on the 3rd and 6th d after administration of a single-dose of PI solution and regeneration began on the epithelial surface, followed by a complete restoration of the epithelial surface on day 10 post administration of iodine (12). Yamamoto et al. (46) indicated that acute inflammatory changes on the superficial layer of the endometrium occurred after the treatment with Lugol solution intrauterinally or PI, and those epithelial cells began to regenerate on the 10th h and completed the regeneration process within 3-4 d.

The authors postulated that this process was similar to that release of prostaglandin from the uterus, and so the recovery process in the uterus occurred much faster. In another study, the intrauterine administration of Lugol, PI, Lorasol, Lotagen, Aureomycin, and saline in 32 healthy cows, degenerative and inflammatory reactions were observed on the first day of administration in all groups, except for those administered with saline. Moreover, similar to our study, the degenerative and inflammatory reactions were highly variable among the cows and were more common in the subepithelial stromal tissues than in the epithelial layers. The regeneration process lasted 3 to 15 d in all groups (35). The lack of degeneration on the surface of the epithelium lamina and the onset of regeneration by day 10 in the literature (35, 46), and the present study (experiment II), may suggest that fertility problems due to postpartum metritis can be treated by PI foam.

In conclusion, it can be stated that, in the cases of retained placenta, the reproductive parameters were unaltered by the treatments, whereas in the case of endometritis, PI foam caused the days to 1st service and days open to be shortened by 13 and 17 d, compared to PGF2α. *E. coli* was the most predominant bacterium in the uterine flora, and *E. coli* and other species totally diminished on day 15 post-administration of PI foam, causing mild pathomorphological changes. Our results suggest that a low dose of povidone iodine in foam form can be used as a therapeutic agent in retained placenta and endometritis.

References


