

## Evaluation of the Treatment of Repeat Breeder Dairy Cows with Uterine Lavage plus PGF<sub>2α</sub>, with and without Cephapirin

Mohammad Rahim AHMADI<sup>1,\*</sup>, Seyed Asghar DEGHAN<sup>2</sup>

<sup>1</sup> Department of Clinical Science, School of Veterinary Medicine, Shiraz University, P.O. Box: 71345-1731, Shiraz - IRAN

<sup>2</sup> Department of Clinical Science, School of Veterinary Medicine, Azad University of Kazeroon - IRAN

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**Abstract:** Repeat breeder syndrome is a major source of economic waste in dairy herds. The purpose of this study was to evaluate the effect of uterine lavage plus PGF<sub>2α</sub>, with and without antibiotic treatment, on the conception rate in repeat breeder dairy cows. The study included 33 repeat breeder cows free of any significant detectable pathologic disorders associated with the reproductive tract. The cows were divided into 2 groups. Group A (n = 10), the treatment group, was lavaged with normal saline, and was administered 2 doses of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) (13 days apart) and an intrauterine infusion of benzathine cephapirin. Group B (n = 23), the control group, was lavaged with normal saline and administered 2 doses of PGF<sub>2α</sub> (as above), but did not receive benzathine cephapirin. All cows were lavaged during the luteal phase (7 ± 2 days after estrus signs). After administration of the second PGF<sub>2α</sub> dose, the cows were inseminated following standing heat. Following the first artificial insemination (AI) treatment, 20 cows (60.6%) became pregnant. Conception rates were 70% (7/10) in group A and 56.52% (13/23) in group B; however, this difference was not statistically significant. The results showed that uterine lavage plus PGF<sub>2α</sub>, without any antibiotic, may be preferable in the treatment of repeat breeder cows. Uterine lavage plus PGF<sub>2α</sub> had some advantages, such as no milk waste or side effects on the endometrium, and may have improved the conception rate, to some extent. Further studies are required to confirm these findings.

**Key Words:** Repeat breeder, uterine lavage, conception rate, endometritis, PGF<sub>2α</sub>, cephapirin

### Introduction

Repeat breeder syndrome is a major source of economic waste in dairy herds. Cows that fail to conceive after a defined number of inseminations with fertile semen (generally 3 or more) are classified as repeat breeders (1); 10%-15% of cows that fail to conceive on time can be classified as repeat breeders (2).

There is increasingly clear evidence that chronic uterine damage in cows results from infection (3). Levine (1) did not place great importance upon chronic uterine infection as a cause of repeat breeding. Sagartz and Hardenbrook (4) reported that 77% of infertile cows had endometritis. In a study by Hartigan et al. (2), 50% of the genital tracts obtained from an abattoir showed histological evidence of endometritis, yet only 12.5% showed gross lesions. Hence, it is likely that subclinical

endometritis is a major contributor to the repeat breeder syndrome of bovine subfertility (3).

As mentioned above, an abnormal uterine environment may cause repeat breeding and endometritis is one of the most important causes; therefore, improvement of the intrauterine environment for embryo survival is the basis of different therapeutic methods. The cellular immune response in the uterus may be adversely affected by several therapeutic strategies, such as intrauterine administration of antiseptics, disinfectants, and antibiotics, which are commonly used to treat postpartum disorders in cattle (5,6).

Veterinarians must identify and treat uterine diseases efficiently in order to limit their negative effects on fertility (7). There has been some interest in finding new therapeutic methods, such as the use of

\*E-mail: rahmadi@shirazu.ac.ir

immunomodulators of the uterine defense mechanisms, as alternatives to the traditional use of intrauterine antibiotics, antiseptics, and disinfectants. They act as chemoattractants and increase the number of polymorphonuclear cells (PMNs) in the endometrium and help to resolve endometritis in cows (8). In a preliminary trial in repeat breeder cows, following intrauterine infusion of *E. coli* and treatment with lipopolysaccharide as an immunomodulator during estrus, a majority of the cows with turbid vaginal discharge conceived (9). The results of that trial might have been due to the clearance of the bacterial infection from the uterine lumen within one estrous cycle (10) and this demonstrates that the clearance of uterine lumen with non-invasive methods, such as the use of prostaglandins in the luteal phase and uterine lavage with normal saline, may have positive effects on the fertility of repeat breeder cows.

Normal saline is the preferred fluid for uterine lavage of mares, because it is mildly irritating to the endometrium, causes cellular response, and is a clear fluid, which allows easy inspection of the recovered material (11).

PGF<sub>2α</sub> has been used in the treatment of endometritis. After prostaglandin treatment, cows come into the estrus phase and the suppressive effect of progesterone on the uterine defense mechanism is removed. Stimulation of myometrial contraction by PGF<sub>2α</sub> may be a mechanism that expels debris and microorganisms from contaminated uterine lumen (12). PGF<sub>2α</sub> may stimulate the phagocytic activity of uterine polymorphonuclear cells as well (13). As a result of its luteolytic action, PGF<sub>2α</sub> should be made the treatment of choice concerning endometritis in cows with a functional corpus luteum present.

The aim of this study was to evaluate the effect of uterine lavage plus PGF<sub>2α</sub>, with or without cephalosporin, on conception rate in repeat breeder dairy cows.

## Materials and Methods

The study included 33 repeat breeder cows (artificial inseminations > 3) with 2 to 5 parturitions that were selected from a Holstein dairy farm in the southeast of Shiraz, Iran. The farm was located at lat 29°51N, long 52°35E, at an elevation of 1500 m above sea level. The body condition score (BCS) was 2.25-3 (scale 1-5) in all cows. Cows were classified as repeat breeders if they

were cycling and showed estrus, had no significant detectable pathologic defect associated with the reproductive tract, and had returned to heat after ≥ 3 services (2). The cows were divided into 2 groups. Group A (n = 10) was treated with saline lavage, 2 doses of PGF<sub>2α</sub> (Lutalyse<sup>®</sup>, Upjohn, Belgium), and intrauterine infusion of benzathine cephalosporin (500 mg) (Metri-care<sup>®</sup>, Afarin Daroo, Iran) (14). Group B (n = 23) was treated with saline lavage and 2 doses of PGF<sub>2α</sub>. All cows were lavaged during the luteal phase of the estrus cycle (7 ± 2 days after estrus signs). Disposable injection pipettes were used to infuse 300-400 ml of normal saline and a repeated dose of 50 to 100 ml.

The head of the pipette was passed through the cervix and 50-100 ml of normal saline, depending on the size of uterine horn, was injected into each uterine horn. The solution flowed out very gently from the uterus through the pipette. This procedure was repeated 4 or 5 times. In 10 cases (group A), Metri-care<sup>®</sup> was infused into the uterus after being lavaged. Following lavage, 2 doses of prostaglandin F<sub>2α</sub> (25 mg of Lutalyse<sup>®</sup>) were administered intramuscularly 13 days apart; the first dose was administered the day of treatment and the other dose was administered 13 days later. Subsequently, cows were inseminated 8 to 12 h after the standing heat. All cows were inseminated by the same person and all the insemination semen (0.5 ml) use was produced by one Iranian company (Center of Animal Breeding, Karj, Iran). Pregnancy diagnoses were performed on day 45 following insemination through rectal examination. For each treatment, the data were analyzed using the chi-square test. A value of P < 0.05 was considered statistically significant.

## Results

Among the 33 treated cows, 20 (60.6%) became pregnant. Among the pregnant cows, 13 (39.4%) received only treatment of uterine lavage and PGF<sub>2α</sub> (group B), whereas 7 cows (21.2%) also received Metri-care<sup>®</sup> (Group A). In group A there were 3 non-pregnant cows (9.1%). In group B, there were 10 non-pregnant cows (30.3%). In all, there were 13 non-pregnant cows (39.4%), but there was no significant difference between the 2 treatment methods (P > 0.05). The conception rate of group A was higher than that of group B (70% vs. 56.52%, respectively) and this difference was not statistically significant (P > 0.05) (Figure).

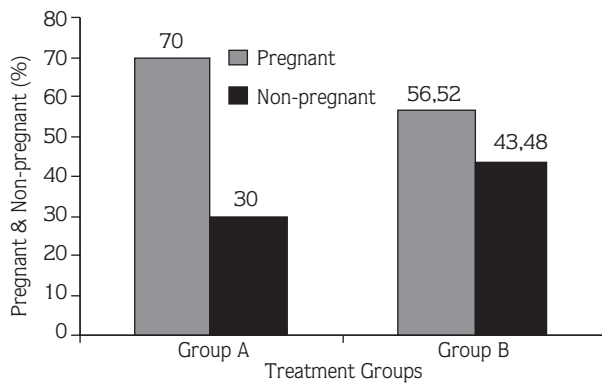


Figure. Pregnancy rate of repeat breeder dairy cows treated with uterine lavage plus PGF<sub>2α</sub> with (group A, n = 10) and without (group B, n = 23) cephalixin (Metri-Care®).

## Discussion

In our study, 60.6% of repeat breeder cows conceived. It seems that many repeat breeder cows are fertile and they should not be culled from herds, especially when they are valuable. The conception rate was 70% (7/10) in the Metri-care group and 56.52% (13/23) in the other one, but this difference was not significant ( $P > 0.05$ ). Repeat breeding is a multifactorial condition and there is no available accurate method for diagnosing the cause of most individual cases. Therefore, the mechanisms by which therapeutic uterine lavage works are not well understood (15). The results of the present study showed that the clearance of the uterus of undesirable conditions for embryonic growth by means of lavage can decrease endometrial inflammation and may cause an increase in the conception rate.

Seitaridis and Tsangaris (16) detected light, moderate, and severe degrees of endometritis (20%, 50%, and 26%, respectively) among 50 repeat breeder cows. Some other researchers reported a high incidence of endometritis in repeat breeder cows (2). Specific and non-specific infectious agents during the pre- and postpartum periods frequently invade the uterus and produce metritis and endometritis, leading to repeat breeding (17). Among the various causes of repeat breeding, bacterial infection is of prime importance (17). In a study by Osman et al. (18), the incidence of bacterial isolation was 42% pure culture and 48% mixed culture. Javed and Khan (17) also observed the same percentages. In a later study by Javed and Khan (17), no sterile case was detected; but Osman et al. (18) reported a 9% incidence of sterile cases.

Endometrial biopsies, before and after uterine lavage of postpartum cows, resulted in reductions of inflammation in the majority of cases in which endometritis was present before lavage (15). These findings suggest that uterine lavage physically removes microorganisms, debris, and possibly anti-sperm antibodies the cow is unable to expel, reducing inflammation and allowing normal sperm/ova transport, conception, and pregnancy to occur (15). Tizard (19) suggested that in chronic bacterial infection the causative bacteria produce certain chemical factors, which inhibits the stimulation of uterine defense mechanisms. Uterine infusion with *E. coli* LPS, as a uterine defense stimulator, can cause infiltration of PMNs into the bovine uterine lumen, which is responsible for the clearance of bacteria from the uterus by phagocytosis. Indeed, Singh et al. (10) observed a 75% conception rate in uterine infusion with *E. coli* LPS. Possibly, one of the reasons for the increase in the conception rate of repeat breeder cows treated with uterine lavage is the clearance of bacteria from the uterus.

Normal saline solution infusion caused no specific alteration in the number and type of cellular infiltrations of the endometria when compared to the control animals; but, after 24 h of the infusion of Lugol's solution and oxytetracycline, the endometria showed the signs of acute inflammatory reaction (20). Fibroblasts also increased at 72 h post-infusion (20). These findings show the advantages of uterine lavage in comparison with other uterine infusion methods (Lugol's and oxytetracycline).

In other studies of infertile cows, some evidence of a high incidence of uterine infection and endometritis was reported (4). Such high incidences of inflammatory states may be due to the use of antibiotics in the treatment of repeat breeder cows. Levine (1), however, did not place great importance upon chronic uterine infection as a cause of repeat breeding.

Treatment of bacterial endometritis, as a major cause of repeat breeding (21,22), with parenteral or intrauterine infusion of antibacterial agents and antibiotics resulted in varying degrees of success (10). Unreliable treatment success, a high cost of treatment, the loss of milk, and the development of microbial resistance to antibacterial drugs are obvious disadvantage of their use. Moreover, antibacterial drugs can also markedly inhibit or destroy the phagocytic activity of PMNs, which are responsible for maintaining uterine

defense (23). Metri-care® is a broad spectrum antibiotic that can be used in intrauterine form. Its withholding period for meat is 2 days and zero for milk (24). Because of its benefits, we used this product in the treatment of repeat breeder cows. Concerning the importance of infectious agents in the etiology of repeat breeder syndrome, the use of an antibiotic, such as cephapirin, for treating these cows is needed. The results of the present study showed that there was no significant difference between the conception rates of the 2 groups ( $P > 0.05$ ) treated with or without cephapirin. The possible reason for this occurrence may have been a decrease in endometrial inflammation and the amount of microorganisms due to uterine lavage. It is likely that it may not be necessary to use any antibiotics after uterine lavage in repeat breeder cows.

The use of PGF<sub>2α</sub> plus uterine lavage reduces the interval from treatment to conception and reduces the possibility of missed heats. PGF<sub>2α</sub> might improve the uterine environment and alter the endocrinal status of repeat breeder cows leading to fertility (25). It limits mild endometritis by increasing phagocytic activity of the

uterine leucocytes and by its stimulatory actions on the smooth muscles of the uterus (25). Further, some physiological and biochemical changes might be associated with alteration in the endometrial vascular permeability (26). In Goley and Kadu's (25) study using PGF<sub>2α</sub> in the luteal phase, the conception rate in cows that received gonadotropin releasing factor (GnRH) and human chorionic gonadotropin (hCG) before their inseminations were 57.14% and 42.80%, respectively. This study showed that PGF<sub>2α</sub> alone might not treat repeat breeder cows as LeBlanc et al. (27) previously reported. Regarding their observations, the cows that received PGF<sub>2α</sub> alone in the luteal phase did not conceive (25,27).

Although the sample size used in the present study was small, it appeared to be sufficient to obtain a sound conclusion. The results of this study imply that uterine lavage plus PGF<sub>2α</sub> has some advantages, such as no milk wastage or side effects on the endometrium, and may improve the conception rate, to some extent. However, further studies are required to confirm these findings.

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