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Effect of suppression of postpartum ovulation on endometrial inflammation in dairy cows

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ABSTRACT

The objective of this study was to investigate the effect of time of first postpartum ovulation on endometrial inflammation in dairy cows with and without uterine disease during the early puerperal period. Transvaginal follicular puncture (FP) was carried out to suppress postpartum ovulation and formation of a CL until Day 42. Fifty-three lactating Holstein Friesian cows were divided into four groups on the basis of presence (UD+) or absence (UD−) of uterine disease, which was defined as retained fetal membranes and/or metritis, and whether FP had (FP+) or had not been (FP−) carried out. This resulted in the following groups: UD−FP− (n = 15), UD−FP+ (n = 13), UD+FP− (n = 13), and UD+FP+ (n = 12). Cloprostenol was given on Days 55 to 60 postpartum, and GnRH was administered 2 days later for synchronization of ovulation. In the FP− groups, endometrial swab and biopsy samples were collected during the second estrus (approximately Day 40) and during the estrus after synchronization. In the FP+ groups, the same samples were collected during the first estrus (approximately Day 49) and during the estrus after synchronization. The prevalence of positive bacteriologic cultures of the endometrium was not affected by FP ($P > 0.05$). Histologic signs of endometritis were more severe in UD+FP− cows at second sampling than in UD+FP+ cows ($P \leq 0.05$). Endometrial expression of *IL1α* (in UD− after first sampling and in UD+ after second sampling) and *IL1β* (in UD− and UD+ after first sampling) was higher ($P \leq 0.05$) in FP− cows than in FP+ cows. Regardless of group, cows with histopathologic evidence of endometritis had higher expression ($P \leq 0.05$) of *IL1α*, *IL1β*, *IL6*, and *TNFα* than cows without endometritis. In conclusion, suppression of early ovulation by transvaginal FP enhances clearance of uterine inflammation in postpartum cows.

1. Introduction

Resumption of ovarian cyclicity postpartum occurs during involution of the reproductive tract and is a precondition for successful breeding [1]. The optimal length of the postpartum anovulatory period is controversial; several studies found that early ovulation had a

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positive effect on reproductive performance [2–5], whereas another study reported reduced fertility [6]. Suppression of early postpartum ovulation by administration of a GnRH analog (deslorelin) [7,8] or by transvaginal follicular puncture (FP) [9] enhanced uterine involution resulting in a reduction in the diameter of the uterus and cervix.

In addition to a decrease in size, uterine involution includes regeneration of the endometrium and resolution of uterine inflammation with elimination of bacterial contamination postpartum [1,10–12]. When clearance of uterine bacterial contamination fails, infection and inflammation persist resulting in chronic clinical or subclinical endometritis [13–15]. Both conditions impact reproductive performance negatively by increasing the number of days open and decreasing pregnancy rates [16–19]. A short postpartum anovulatory period appears to impair elimination of uterine bacterial contamination and inflammation. Experimental suppression of postpartum ovulation resulted in a lower frequency of purulent vaginal discharge compared with cows that ovulated early in the postpartum period [7,9]. Failure to eliminate uterine inflammation was largely attributed to the immunosuppressive effect of progesterone (P_4) produced by the CL [6,7,9].

A diagnosis of clinical endometritis is based on the presence of endometrial inflammation accompanied by purulent vaginal discharge after Day 21 postpartum. Subclinical endometritis is characterized by an increase in neutrophil numbers in endometrial cytobrush samples and absence of overt vaginal discharge [20]. The cytobrush technique is a routine diagnostic tool for the detection of subclinical endometritis [17,21,22]. However, a recent study showed no agreement between the cytologic results of endometrial cytobrush samples and histologic findings of endometrial biopsy samples in cows with subclinical endometritis [23]. Chronic endometritis is characterized histologically by large numbers of lymphocytes and neutrophils, particularly in the stratum compactum [24]. However, these cells are not necessarily found in the uterine lumen and therefore may not be collected using the cytobrush method [23].

Leukocytic infiltration and increased expression of proinflammatory cytokines are normal components of endometrial inflammation in the early puerperal period and play an important role in bovine uterine health [12,13,25]. An increase in the gene expression of endometrial cytokines, including tumor necrosis factor α (TNFα) and interleukin (IL) 1 and 6, occurs in clinical and subclinical endometritis [15,21,26,27]. Interleukin 1α, which is released during cell necrosis in acute inflammation, mediates neutrophil recruitment and induces release of IL1β and other cytokines [28]. Tumor necrosis factor α and IL1β play a critical role in infectious disease processes by stimulating macrophages, T cells, other cytokines, and chemokines [29,30]. Interleukin 6, produced by macrophages, also activates T cells and promotes immunoglobulin secretion [30,31]. The expression of proinflammatory cytokines in the bovine endometrium has only been studied in superficial endometrial epithelial cells collected with a cytobrush [21,26,27]. To the authors' knowledge, the effect of the time of first postpartum ovulation on histologic and molecular inflammatory parameters of the endometrium has not been investigated in cows.

Thus, the objective of the present study was to investigate the effect of suppression of early postpartum ovulation on the histologic inflammatory response of the uterus and on endometrial proinflammatory cytokines in healthy cows and in cows with postpartum uterine disease.

2. Materials and methods

2.1. Animals

This study was conducted from February 2009 to July 2010 using the dairy herd at the Research Farm of the University of Veterinary Medicine Hannover, Germany. The study was approved by and carried out in accordance with German legislation on animal rights and welfare (33.9-42502-04-08/1592). The herd consisted of 90 milking cows with a rolling herd average of 10,000 kg of milk per lactation. The cows were housed in a freestall barn, had free access to water, and were fed a mixed ration supplemented with concentrates based on milk yield.

2.2. Study design

Cows were assigned to two groups depending on whether uterine disease was present (UD+) or absent (UD−). Uterine disease was defined as metritis and/or retention of fetal membranes for more than 24 hours. Metritis was diagnosed between Days 4 and 21 postpartum (Day 0 = day of calving) according to the criteria described by Sheldon et al. [20]. Both groups were randomly subdivided into two subgroups: in the first, ovulation was suppressed by transvaginal FP (FP+) until Day 42 and in the second, the time of ovulation was not manipulated (FP−). This resulted in four groups designated UD−FP−, UD−FP+, UD+FP−, and UD+FP+. Cows in the FP− group that did not ovulate by Day 42, cows in the FP+ group with a P_4 concentration greater than 1.0 ng/mL before Day 42, and cows with other organ system diseases were excluded from the study.

A general physical examination, transrectal and vaginal palpation, and B-mode sonography of the reproductive tract were conducted on Days 8, 11, 18, 25, and then every 10 days until Day 65. Blood sampling for estimation of P_4 was done weekly. Transvaginal FP was carried out in cows of the FP+ groups as published earlier [9]. For synchronization of ovulation, all cows received 0.5-mg cloprostenol (intramuscular; Estrumate; MSD, Unterschleißheim, Germany) between Days 55 and 60 and 0.01-mg GnRH (intramuscular; buserelin; Receptal, MSD) 2 days later. In the FP− groups, endometrial sampling (ES; swab and biopsy) was conducted during the second estrus (approximately Day 40) and during estrus after synchronization (ES2). In the FP+ groups, ES was carried out during the first estrus (ES1; approximately Day 49) and during the estrus after synchronization (ES2).

2.3. Bacteriologic examination

Uterine swabs were collected for bacteriologic culture using a sterile cytobrush (Cytobrush Plus GT; Medscand