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Low plasma progesterone concentrations are accompanied by reduced luteal blood flow and increased size of the dominant follicle in dairy cows

J. Lüttgenau[a,*], N. Beindorff[a], S.E. Ulbrich[b], J.P. Kastelic[c], H. Bollwein[a]

[a] *Clinic for Cattle, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany*
[b] *Physiology Weihenstephan, Z I E L Research Center for Nutrition and Food Sciences, Technical University of Munich, Weihenstephaner Berg 3, 85350 Freising–Weihenstephan, Germany*
[c] *Agriculture and Agri-Food Canada, Lethbridge Research Center, Lethbridge, AB, T1J 4B1 Canada*

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Abstract

To investigate the influence of low plasma progesterone (P_4) concentrations on luteal and ovarian follicular development as well as endometrial gene expression in the concomitant and subsequent estrous cycle, 20 lactating dairy (Holstein Friesian and Brown Swiss x Holstein Friesian) cows received either a single treatment with 25 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on Day 4 Hour 12 (PG1; n = 8), or two treatments (25 mg $PGF_{2\alpha}$ each) on Day 4 Hours 0 and 12 (PG2; n = 12) of the estrous cycle (Day 1, Hour 0 = ovulation). In four cows, ovulation occurred between 4 and 6 d after the second $PGF_{2\alpha}$ treatment; these cows and one lame cow were excluded. In the 15 remaining cows with physiological interovulatory intervals (18 to 24 d), P_4, luteal size (LS) and blood flow (LBF), as well as follicular size (FS) and blood flow (FBF), were determined daily until Day 4, immediately prior to (0 h) and 12 h after each $PGF_{2\alpha}$ treatment, and then every 2 d, from Day 5 to 8 d after the subsequent ovulation. Because P_4 did not differ ($P > 0.05$) between PG1 and PG2, cows were regrouped according to their mean P_4 concentration from Days 7 to 15, either P_4 <2 ng/mL (P_4L; n = 7) or P_4 >2 ng/mL (P_4H; n = 8). In the treatment cycle, LS was smaller in P_4L than P_4H on Days 13 ($P = 0.01$) and 15 ($P = 0.03$), and LBF was lower in P_4L than P_4H on Day 15 ($P = 0.02$). The dominant follicle of the first follicular wave was larger in P_4L than P_4H on Days 13 ($P = 0.03$), 15 ($P = 0.03$), and 17 ($P = 0.01$). In the subsequent cycle, there were no significant differences between P_4L and P_4H for P_4, FS, LS, and LBF; however, FBF was lower ($P = 0.01$) in P_4L than P_4H on Day 7. In Group P_4L, endometrial expressions of estrogen receptor α and oxytocin receptor were lower ($P = 0.05$ and $P = 0.03$, respectively) at the estrus that preceded treatment compared to the post-treatment estrus. In summary, low P_4 during diestrus was associated with smaller LS, reduced LBF, and larger FS in the treatment cycle, but not in the subsequent cycle.

Keywords: Low progesterone; Corpus luteum; Luteal blood flow; Follicular size; Bovine

1. Introduction

Dairy cow fertility has declined in association with increased milk production, apparently due in part to increased embryonic mortality [1,2]. There are numerous causes for embryonic mortality; low systemic progesterone (P_4) concentrations, not only during early pregnancy [3], but also during the estrous cycle preceding insemination [4], were associated with an increased incidence of embryonic loss.

Low P_4 concentrations have an important influence on luteal as well as follicular development in cattle. In a previous study [5], low P_4 in dairy cows was specu-

* Corresponding author. Tel.: +49 511 856 7222; fax: +49 511 856 7693.
E-mail address: johannes.luettgenau@tiho-hannover.de (J. Lüttgenau).

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lated to interfere with luteal maintenance in the subsequent cycle, due to its stimulatory effect on uterine secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Furthermore, persistent large follicles with aged (prematurely activated) oocytes developed under low systemic P_4 concentrations [6,7], and in a more recent study, low P_4 increased the diameter of the preovulatory follicle, as well as the resulting corpus luteum (CL) [8]. Therefore, effects of low P_4 concentrations on the CL of the subsequent cycle were either exerted directly by stimulating uterine secretion of luteolytic $PGF_{2\alpha}$ [5], or indirectly by increasing the diameter of the preovulatory follicle [6–8].

Angiogenesis and hemodynamic changes are involved in cyclic remodelling of both the follicle and CL [9,10]; therefore, measurement of ovarian perfusion should provide valuable information regarding follicular and luteal function. However, until recently, measurement of ovarian perfusion required complex, invasive instrumentation [11]. Approximately two decades ago, color Doppler sonography was introduced in human gynecology for noninvasive investigation of ovarian dysfunction [12]. Reduced perfusion of either the follicle or the CL was associated with reduced pregnancy rates in humans [13,14]. In recent studies, color Doppler sonography was used to study ovarian perfusion in cattle. Whereas reduced blood flow to the preovulatory follicle was associated with decreased pregnancy rates in cattle [15], luteal blood flow (LBF) was studied only during luteal development [16,17], after induced luteolysis [16,18], or during early pregnancy in cattle following transfer of an embryo [19].

Sufficient P_4 concentrations are required for the remodelling of the uterus into an adequate environment to support embryonic development [20]. However, the molecular mechanisms by which low P_4 concentrations impair uterine function and interfere with embryonic development have not been clearly elucidated. A recent study [21] investigated the effect of P_4 supplementation during the early luteal phase (Days 4 to 8 post-ovulation) on endometrial expression (Day 8) of progesterone receptor (PGR) and estrogen receptor α (ESR1). Increasing P_4 concentrations were associated with decreased expression of both PGR and ESR1. However, to our knowledge, the influence of low P_4 concentrations during the mid- and late-luteal phases on endometrial gene expression at the following estrus in nonpregnant cows has not been reported.

In addition to physical removal of the CL or CL-bearing ovary [22], $PGF_{2\alpha}$ treatment is a noninvasive method to reduce endogenous P_4 secretion. Although the CL was refractory to the luteolytic effect of $PGF_{2\alpha}$ during the first 4 d of the estrous cycle [23], repeated $PGF_{2\alpha}$ treatments during this time reduced P_4 concentrations during diestrus [24–26]. For example, a single $PGF_{2\alpha}$ treatment 4 d after estrus had no effect on plasma P_4 concentrations, whereas two treatments, 12 h apart, reduced P_4 concentrations approximately 50%, without affecting the length of the estrous cycle [24]. Therefore, we expected this would be a useful model to reduce peripheral P_4 concentrations without altering CL lifespan.

The primary objective of the present study was to determine the effects of low plasma P_4 concentrations on luteal and follicular development in the concomitant estrous cycle (following treatment with $PGF_{2\alpha}$), and in the subsequent (untreated) cycle. We tested the hypotheses that low P_4 concentrations during diestrus were associated with increased diameter of the dominant follicle and decreased blood flow in the wall of the dominant follicle, as well as within the CL. Furthermore, a secondary objective was to determine the effects of low P_4 concentrations during diestrus on gene expression of endometrial receptors for P_4, estrogen, oxytocin, and $PGF_{2\alpha}$ (at the following estrus).

2. Materials and methods

2.1. Cattle

Twenty primi- and pluriparous (n = 6 and n = 14, respectively), lactating Holstein Friesian (n = 14) and Brown Swiss x Holstein Friesian (n = 6) cows were used. These cows were (mean ± SEM) 3.3 ± 0.3 yr old, with body weight 570.9 ± 12.7 kg, body condition score (BCS) 2.9 ± 0.1 (scale, 1–5), and daily milk production 36.3 ± 1.6 kg. Starting immediately after calving, cows were examined twice weekly by transrectal palpation and ultrasonography of the uterus and ovaries (the latter were not examined during the first 2 wk, as they were not accessible). Only cows that were clinically healthy, with no apparent reproductive abnormalities, and a first postpartum ovulation within 3 wk after calving, were used. Before the start of this study, every cow had at least one estrous cycle (CL detected for at least three consecutive examinations, based on a twice-weekly examination schedule). The cows were housed in a freestall barn at the Research Farm of the University of Veterinary Medicine Hannover and fed a total mixed ration (primarily silage and concentrate), with *ad libitum* access to water. The experimental protocol was approved by the Ethics Committee on Animal Rights Protection (Oldenburg, Germany), in accor-