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Pregnancy outcome is influenced by luteal area during diestrus before successful insemination but not by milk production level

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ABSTRACT

The objective was to compare luteal area (LA), luteal blood flow (LBF), and progesterone (P4) concentration before and after artificial insemination (AI) in pregnant and open cows in the diestrus preceding insemination and during the first 21 d after insemination. A number of 119 multiparous German Holstein cows were examined using B-mode and color Doppler sonography in diestrus (15–5 d before AI) and on days 7, 14, and 21 post insemination (pi). Blood samples for determination of P4 concentration were collected at each examination. In pregnant cows, P4 was measured in the diestrus before AI and on days 7, 14, and 21 of pregnancy, and in open cows, in the diestrus preceding the first AI and 7, 14, and 21 d later. Cows were retrospectively allocated into two groups according to the mean daily milk yield in the first 42 d (high milk yield [HMY], ≥39 kg/d; low milk yield [LMY], <39 kg/d). Pregnant cows had larger LA than open cows during diestrus before insemination (5.69 ± 1.85 vs. 4.94 ± 1.66 cm^2, $P < 0.05$), on days 7 (5.34 ± 1.83 vs. 4.52 ± 1.93 cm^2, $P < 0.05$) and 21 pi (5.92 ± 1.60 vs. 4.97 ± 1.44 cm^2, $P < 0.05$). On day 14 there was a tendency towards larger LA in pregnant cows ($P = 0.09$). Luteal blood flow (1.72 ± 0.74 vs. 1.22 ± 0.67 cm^2) and P4 concentration (8.97 ± 4.37 vs. 6.49 ± 4.32 ng/mL) were higher in pregnant cows than in open cows on day 21 ($P < 0.05$). At day 150, 69% of the LMY cows and 56% of the HMY cows were pregnant ($P > 0.05$). Pregnant HMY cows had larger LA during diestrus before insemination and on days 7 and 21 pi than open HMY cows ($P < 0.05$) and tended to result in larger CLs on day 14 ($P = 0.06$). Pregnant HMY cows had higher LBF on days 14 and 21 ($P < 0.05$) and higher P4 concentration on day 21 than open HMY cows ($P < 0.05$). The main finding of this study was a larger LA during diestrus in cows that conceived in the ensuing estrous cycle compared to cows that did not conceive. Luteal area, LBF, and P4 concentration were greater in pregnant cows compared to open cows, and further studies should be conducted on the usefulness of these variables to detect open cows as early as possible after breeding. High-yielding cows appear to require larger LA, LBF, and higher P4 concentration than low-yielding cows to maintain pregnancy. In conclusion, larger LAs were established in pregnant cows in the diestrus before successful insemination regardless of their milk yield. Further studies are needed to verify whether LA in the diestrus before insemination might be used to predict probabilities in reproductive performance.

1. Introduction

Establishment and maintenance of pregnancy in cattle depend strongly on an active, functional corpus luteum (CL) that produces a sufficient amount of progesterone (P4; [13,16,18]). A rapid rise in the blood P4 concentration starting at approximately day 5 post insemination (pi) is necessary for normal embryonic development. Interestingly, a rapid increase in P4 appeared more critical for the establishment and maintenance of pregnancy than the P4 level itself [15]. Blood P4 concentration increased about 4.8-fold from day 3–13 after conception and remained unchanged thereafter until day 21 [9]. Pregnant cows had higher P4 concentrations on day 9 after ovulation than open cows [8,9]. Timed artificial insemination (AI) after ovulation synchronization (OvSynch) protocols resulted in lower pregnancy rates in cows that had P4 serum concentrations < 1 ng/mL during diestrus 10 d before insemination [3].

Embryonic loss increases days open and may occur because of insufficient blood P4 levels. High-producing dairy cows have an enormous energy demand, and an efficient blood flow from the

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gastrointestinal tract to the liver is required to satisfy this demand. The liver metabolizes nutrients as well as steroid hormones such as P4 and estrogen. It was hypothesized that high-yielding cows compensate the increased metabolism of P4 and estrogen by developing larger CLs and follicles [20,21,26]. Studies that investigated the correlations between luteal area (LA) and luteal blood flow (LBF) and between LA and P4 concentration in cycling cows [7,14] led to the conclusion that LBF and P4 concentrations reflect luteal function more reliably than LA [6,7].

Pregnant cows had larger LA than open cows on days 15 and 18 pi [8]. Likewise, luteal volume was larger and LBF greater in pregnant cows than in open cows on days 17, 19, and 20 pi [24]. A decrease in LBF occurred in open cows on day 19 pi whereas LBF did not change significantly in pregnant cows from day 16 to day 23 pi [17]. Likewise, LBF remained unchanged in pregnant cows and in cows with embryonic loss but was reduced significantly on days 15 and 18 pi in open cows [8]. A decrease in LBF at the time of suspected luteolysis in inseminated cows was considered a useful tool for the early diagnosis of open cows rather than for early pregnancy diagnosis [19,23,24].

The primary goal of this study was to investigate LA, LBF, and P4 concentration in the diestrus preceding insemination and during the first 21 d after insemination to predict pregnancy. A secondary goal was to examine the effect milk production on luteal morphology and activity by comparing these variables between cows producing <39 and ≥39 kg milk/d in early lactation.

2. Materials and methods

2.1. Animals

The study was conducted from September 2013 to August 2014 and involved 144 German Holstein cows from a 400-cow dairy herd in Lower Saxony, Germany. Twenty-five cows were excluded from the study because of dystocia, twinning, displaced abomasum, lameness or toxic mastitis, which left 119 cows for analysis. Of these, 56 (47%) were in 2nd, 22 (18%) in 3rd and 41 (35%) greater than 3rd lactation, respectively. Dry cows were housed in a free stall with cubicles and straw-manure bedding. In the last two weeks of gestation, cows were fed the same ration as the high-producing cows, which based on 30 kg of milk production. The ration contained grass and corn silage, alfalfa hay, potato pulp, ensilaged brewers grains, bakery meal, a mixture of grain and minerals. Groups of four cows were kept in straw-bedded calving pens, where they remained after calving for up to three days. They were then moved to the group of high-producing cows in the free stall barn. Concentrate was fed individually at transponder stations, and the daily amount was increased gradually to 7 kg on day 24. Thereafter, the amount was adjusted to production and the cows received one extra kg for every 2 kg of milk exceeding 30 kg. Cows were milked twice daily between 5 and 8 a.m. and between 4 and 7 p.m. in a rotatory herringbone parlor milking system HBR from DeLaval GmbH (Glinde, Germany). High-producing cows were milked first in the morning and last in the evening. Daily milk yield was recorded using the ALPRO™ herd management system from DeLaval GmbH (Glinde, Germany). For analysis, cows were classified as high or low producing depending on mean daily milk yield in the first 42 d (high milk yield [HMY], ≥39 kg/d; low milk yield [LMY], <39 kg/d). Visual heat detection and artificial insemination were carried out by the American Breeders Service (ABS, Hannover, Germany). Veterinarians from the Niedersachsen Extension Service (Oldenburg, Germany) carried out a reproductive examination between days 10 and 14 postpartum (pp). Depending on the reproductive health of the cows, they received veterinary treatment or were treated by the herd manager. Each cow received prostaglandin (PG) $F_{2\alpha}$ (Dinolytic®, Dinoprost-Trometamol, Pfizer, Germany) on day 21 pp. Cows with low-grade endometritis received another $PGF_{2\alpha}$ injection on day 35 pp. Cows with RFM were treated with ten uterine boluses (Tetra-Sleecol®, 2000 mg, active ingredient tetracycline hydrochloride, Albrecht GmbH, Germany) on day 4. Lochiometra was treated in the same way once or twice 3 d apart. Score 2 and 3 endometritis was treated with uterine infusion of 100 mL Mastipen®comp. (benzylpenicillin-procaine 1 H2O 16.00 mg, benzylpenicillin-potassium 2.50 mg, streptomycin sulfate (Ph.Eur.) 19.44 mg, Medistar Arzneimittelvertrieb GmbH, Germany).

2.2. Study design

Cows were examined clinically and with B-mode and color Doppler ultrasonography on days 7, 14, 28, 42, and 56 pp. Cows that were not inseminated by day 56 underwent a sonographic examination every 10 d until they were bred. After day 43, cows were inseminated in spontaneous estrous cycles or they underwent an OvSynch protocol and fixed-time AI. Ultrasonographic examination of the ovaries was also conducted 7, 14, and 21 d after AI. Cows were not examined on day 21 pi when they returned to estrus and were re-inseminated. Cows underwent ultrasonographic pregnancy examination on day 26, and in pregnant cows the vitality of the embryo was confirmed on day 42. Open cows were examined clinically and with color Doppler sonography on day 42 pi and then every 10 d until the next insemination. Examinations were discontinued on day 42 pi when a cow was confirmed pregnant or on day 150 pp in cows that failed to conceive.

2.3. Reproductive examination

The uterus was examined transrectally by manual palpation [4]. Size, symmetry, content, and contractility were assessed to exclude uterine pathologies. The ovaries were palpated and examined sonographically and the area of the CL and follicles was measured.

2.4. Determination of blood progesterone concentration

At each clinical and sonographic examination, blood samples were collected from the coccygeal vessels into evacuated tubes (Vacuette® system, Greiner Bio-One GmbH, Frickenhausen, Germany) for P4 determination. The clotted blood was centrifuged (Heraeus-Sepatech-Megafuge-3.0R, Hanau, Germany) at 3500*g* at 10 °C for 10 min and the serum was transferred into 2 mL tubes (Eppendorf AG, Hamburg, Germany) and stored at −18 °C for analysis at the Laboratory for Endocrinology of the Clinic for Cattle (University of Veterinary Medicine Hannover). Progesterone concentration of four blood samples was measured in all cows. In pregnant cows, a sample collected in diestrus preceding conception and samples from days 7, 14, and 21 of pregnancy were analyzed. In open cows, a sample collected in diestrus before the first insemination and samples from days 7, 14, and 21 after unsuccessful insemination were analyzed. The samples collected in diestrus were identified retrospectively; samples from 15 to 5 d before AI were selected for analysis. When more than one sample was available for that period of time, the sample accompanied by the larger LA and higher LBF was chosen. The terms “pregnant” and “open cows” refer to the pregnancy status on day 150 pp. A commercial kit (DRG Instruments GmbH, Maribor, Germany) based on a solid-phase competitive enzyme-linked immunosorbent assay (ELISA) was used for P4 measurement. The microtiter plate wells of the kit were coated with a polyclonal antibody against P4. The serum sample was added first to the microtiter wells and then the progesterone-horseradish-peroxidase conjugate. After incubation