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Daily lying behavior of lactating Holstein cows during an estrus synchronization protocol and its associations with fertility

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ABSTRACT

Objectives were to quantify lying behavior (LB) during an estradiol and progesterone-based synchronization protocol, to assess risk factors for ovulation, pregnancy per AI (P/AI), and degree of behavioral change at estrus, and to investigate the associations between estrus LB and walking activity. Holstein cows (43.6 ± 11.0 kg of milk/d) were fitted with leg-mounted accelerometers. Total lying time/d (L_time), bout frequency (bout_N), average lying bout duration, and relative increase in walking activity (ACT%) were evaluated for 1,411 timed artificial insemination events. The day with lowest L_time or bout_N among d −2, −1, and 0 (day of timed artificial insemination) determined the day of behavioral estrus. The variables L_time% and bout_N% represent relative ratios between lowest L_time and baseline (d −7), L_time, and lowest bout_N, and baseline (d −7) bout_N, respectively {e.g., (lowest L_time/baseline L_time) × 100]. Correlation coefficients between L_time% and bout_N% and ACT% were −0.38 and −0.31, respectively. Estrus LB change was considered large if <75% of baseline and small if ≥75% of baseline for both L_time% and bout_N%; average lying bout duration did not change with estrus. Lowest L_time% and bout_N% corresponded to, respectively, 65 ± 21% (mean ± standard deviation; 447 ± 157 min/d) and 65 ± 24% (8.5 ± 4.0 bouts/d) of baseline. The change in L_time% at estrus was smaller when cows had milk yield above average; the change in bout_N% was smaller among multiparous cows and for estrus occurring in the colder season. Likelihood of ovulation was greater when there was larger change in L_time% [odds ratio = 4.9; ovulation rate = 93 (large change) and 76% (small change)], as well as when a corpus luteum was present at start of protocol (odds ratio = 3.6; in the model with L_time%). Likelihood of pregnancy at d 32 was 1.6 times greater for estrus with large change in LB [L_time% or bout_N%; P/AI = 34% (large change in L_time%) and 26% (small change in L_time%)]. Among estrus events with ACT% ≥300% (high intensity), classification by small or large L_time% did not influence P/AI at 32 d. The magnitude of LB change at estrus and its association with fertility suggest potential application toward improved use of activity monitors (e.g., increased estrus detection, fertility prediction). The contribution of LB to accuracy of estrus detection when physical activity is known remains to be addressed. The relationship between intensity of estrus expression and fertility requires further investigations of its physiological rationale and on-farm applications.
Key words: automated detection, estrus intensity, fertility, lying behavior

INTRODUCTION

Current precision dairy farming technologies allow for automated detection of estrus using tools that record different parameters of animal behavior. In a review of the literature, Rutten et al. (2013) found 48 types of sensors applied to fertility management (e.g., estrus detection), where 61% measured aspects of cow activity. Restlessness is an important component of behavioral estrus (Roelofs et al., 2010). Although subjective if visually evaluated, restlessness can be quantified through measurements of steps, neck movements, lying time, and bout frequency, for example. Estrus walking activity and neck movements have been researched regarding patterns (Løvendahl and Chagunda, 2010; Valenza et al., 2012) and associations with environmental and cow factors, endocrine profiles, ovulation timing, and fertility (López-Gatius et al., 2005; Stevenson et al., 2014; Madureira et al., 2015; Aungier et al., 2015). Lying behavior, on the other hand, is more frequently employed in cow comfort and welfare assessments (von Keyserlingk et al., 2012; Charlton et al., 2014). Even though reports of its application toward estrus

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detection are rare (e.g., see Rutten et al., 2013), lying behavior has recently been deemed as a useful predictor of estrus (Dolecheck et al., 2015). Research from our group reported a decrease in lying time of 36% at the day of estrus in nulliparous animals (Silper et al., 2015a).

Expression of estrus (compared with absence of it) at the moment of timed AI (**TAI**) has been associated with greater pregnancy per AI [**P/AI**; Cerri et al., 2004 (Heatsynch protocol); Souza et al., 2007 (Ovsynch protocol with injection of 1 mg of estradiol 17-β 8 h before the last GnRH)]. Among cows that expressed estrus, those with a higher degree of intensity as measured by automated activity monitors (**AAM**) were associated with greater P/AI compared with poorly expressed events (Madureira et al., 2015). Pregnancy per AI was also greater among cows with confirmed ovulation after TAI (Pereira et al., 2014). Estrus expression and fertility likely share endocrine regulators. Possible mechanisms could involve progesterone exposure and the regulation of endometrial estradiol receptors α (Spencer et al., 2004), LH concentration and follicular growth (Cerri et al., 2011a; Cerri et al., 2011b), in addition to plasma estradiol concentration.

Estradiol and progesterone-based protocols are more likely to induce expression of estrus when compared with GnRH-based protocols (Pereira et al., 2013), providing an opportunity to study the associations between intensity of estrus and fertility under controlled conditions. Automated activity monitors allow grading of behavioral estrus, measurements that can be further used as real-time predictors of fertility and reproductive states. The objectives of our research were to measure the change in lying behavior during an estradiol and progesterone-based synchronization protocol and to assess risk factors associated with the degree of behavioral change at estrus, ovulation, and P/AI. In addition, we aimed to investigate the relationship between measurements of activity and lying behavior. Given the knowledge of increased physical activity at estrus, we hypothesized that lying behavior at estrus would be characterized by reduced lying time and bout frequency and would be negatively correlated with activity. We also hypothesized that the magnitude of change in lying behavior would be associated with ovulation rate and P/AI, similarly to previously reports, when estrus intensity was determined by physical activity.

MATERIALS AND METHODS

Cows, Housing, and Management

This study took place at a commercial dairy (São Paulo, Brazil) between July 2013 and June 2014. At the time of the experiment, the herd had an average of 1,700 lactating cows and 305-d average yield of 11,438 kg. Cows were housed in a cross-ventilated freestall barn in groups of 300 animals and milked 3 times daily (at approximately 0500, 1300, and 2100 h). The barn had grooved concrete floors and 2 rows of deep sand-bedded stalls. Fresh TMR balanced to meet or exceed the nutritional requirements of lactating dairy cows producing 40 kg of 3.5% FCM per day (NRC, 2001) was provided thrice daily. Water and TMR were available for ad libitum intake. Experimental procedures followed requirements and practices outlined in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). Procedures were performed while the cows were restrained in headlocks at the feed bunk after the first daily milking.

Synchronization Protocol, Exams, and Blood Sampling

Eligible cows (first AI or after negative pregnancy diagnosis, determined apt for breeding by the herd veterinarian) were enrolled onto an ovulation synchronization protocol according to Pereira et al. (2015; Figure 1). Timed AI was done using commercial frozen-thawed semen. Ovaries were examined by ultrasonography at d −11 (presence or absence of corpus luteum; **CL**), 0 (assessment of largest preovulatory follicle), and 7 (presence or absence of CL to confirm ovulation). Positive pregnancy diagnosis required presence of amniotic vesicle with viable embryo (visible heartbeat). Body condition score (1 to 5 scale at 0.25 increments; Wildman et al., 1982) was recorded at TAI. Milk production was recorded at each milking (AfiLite, Kibbutz Afikim, Israel).

Blood samples were collected at TAI by puncture of the medial coccygeal artery or vein into 10-mL nontreated Vacutainer tubes (BD, São Paulo, Brazil), placed on ice, and centrifuged at 3,000 × g at 4°C for 30 min. Serum was harvested and stored in microtubes at −20°C until analysis. Progesterone concentration was determined using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Intra- and interassay coefficient of variation were, respectively, 5.1 and 5.2%. The minimum detectable concentration was 0.1 ng/mL.

Behavioral Data

All cows carried an accelerometer (Afimilk Pedometer Plus Tag, Afimilk, Kibbutz Afikim, Israel) attached to 1 of the hind limbs within 1 wk of calving. Data were recorded in 2-h time bins, downloaded thrice daily by an electronic scanner at the milking parlor's entrance.