Estrus: Association with Production Parameters and Implications on Fertility

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Take Home Messages

- Information obtained from activity monitors is useful.
    - Milk production, estradiol and follicle diameter are not as correlated with estrus as initially expected.
    - Intensity of estrus is closely associated with fertility.
    - Expression of estrus and its intensity can affect artificial insemination (AI) and embryo transfer success.
- Reproductive programs with strong reliance on estrus detection are highly efficient.
    - Combination with timed-AI is still necessary.
    - Expect more variability among farms.

Next Steps

- Refine estrus-based reproduction programs.
    - Voluntary waiting period, types of protocols, selective synchronization.
- Improve knowledge related with estrus detection, behaviour and ovulation timing.
    - Standing, lying and rumination data.
    - Different sensors, analyses of multiple sensors.
- Genetic selection.

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- Collection of correct phenotype for genomics.
- Individual variation and association with body condition, parity and milk production.

Estrus

The most recent studies have shown that not only proestrus length or estradiol concentrations during estrus affects reproductive tissues, but also the actual display of estrous behaviour seems to have a profound effect on fertility (Madureira et al., 2015a,b). Most of the data currently available in dairy cows on the effect of proestrus and estradiol pertains to the manipulation of the timing of luteolysis and ovulation induction, therefore modifying the proestrus only. Studies that decrease follicular dominance length (Cerri et al., 2009), increase concentrations of progesterone during diestrus (Cerri et al., 2011), proestrus length (Mussard et al., 2003), and production parameters (e.g. lactation and age; Sartori et al., 2002) have shown positive effects on fertilization, uterine environment, and embryonic development (Ribeiro et al., 2012). However, in spite of marked effects related with the afore mentioned modifications of the estrous cycle, not much emphasis has been placed on the isolated or additive analyses of the effect of expression of estrus (within a variety of different treatments) on reproductive tissues. The effect of estrus on fertility will be discussed in the last section of this manuscript, but it is clear that estrus has a positive impact on fertility. Moreover, this effect also seems to be associated with the intensity of estrus, which collectively leads us to questions regarding the detailed physiological mechanisms associated with this improvement in fertility associated with estrus.

In order to answer some of these questions, we aimed to investigate the association of estrus expression at the time of AI with the expression of critical genes in the endometrium, corpus luteum (CL) and embryo during the pre-implantation period (Davoodi et al., 2016). In addition, we evaluated the difference in estrus expression for reproductive parameters such as CL volume, conceptus size, concentration of progesterone in plasma, and follicle diameter. Evidence from this study supports our hypothesis that estrus expression positively influences the expression of target genes important for embryo survivability. Cows that expressed estrous behaviour near AI had a significant improvement in the profile of endometrium gene expression critical for suppressing the local maternal immune system and likely improving adhesion between endometrium epithelial cells and conceptus, as well as partly inhibiting the mRNA machinery for prostaglandin (PG) synthesis. Genes related to immune system and adhesion group in the endometrium were also significantly affected by concentration of progesterone in plasma on day 7. Results from the gene analysis of the CL also confirmed down-regulation of cellular pathways associated with apoptosis and prostaglandin synthesis, which favors CL maintenance and secretion of progesterone, both key to sustain pregnancy (Davoodi et al., 2016). Moreover, cows that displayed estrus yielded longer conceptuses, which is associated with better chances of survival. The effects of expression of estrus seems to interact with progesterone concentration on d 7 of the estrous cycle in a way that positively influences endometrium receptivity and embryo development. The specific causes that lead to the presence or absence of estrus expression are unknown based on the data collected in this study (Davoodi et al., 2016) and warrant further investigations. The expression of estrus can indicate the state of sensitivity of the hypothalamus to estradiol and perhaps the best timing for the optimal function of all other reproductive tissues related with the survivability of the early embryo.

▪ Production Parameters and Expression of Estrus

The detection of estrus in confined dairy cows became a greater challenge as milk production increased. Previous studies that took into account only mounting behaviours as a measure of intensity and duration of estrus have consistently recorded a decrease in this behaviour as milk production increased (Lopez et al., 2004; Rivera et al., 2010). A major question still unanswered is if mounting behaviour can be used as a gold standard for estrus expression (i.e. intensity and duration), considering the challenges faced by dairy cows in free stall barns with concrete flooring that leads to significant physical stress on foot and legs. The estrus detection rate in a recent survey (Denis-Robichaud et al., 2016) was below 50%, but the proportion of cows truly bred upon estrus detection is still unclear as these data are confounded by timed-AI (TAI) use. This extensive failure to submit cows for AI has a major impact on the pregnancy rate of Canadian herds, but also indicates a unique window of opportunity to improve fertility.

Parity, Milk Production and Body Condition

A large field study (Lopez-Gatius et al., 2005) described that the two main factors affecting estrus activity increase were lactation number and milk production, whereas the degree of activity increase was positively correlated with fertility after AI. The authors did not clearly state the latter, but it was recently corroborated in a study by Madureira et al. (2015a). Milk production, for example, seems to affect the overall sensitivity of pedometers or activity monitors to detect true events of estrous behaviours. However, none of the previous studies measured detailed reproductive physiological events associated with natural estrous behaviour and the level of activity of automated activity monitor (AAM) systems associated with those events. Just recently, more robust studies using adequate numbers of observations of estrus and cows have been published for more reliable conclusions.

A recent study by our group identified several risk factors associated with the intensity of estrus expression. Multiparous Holstein cows expressed lower peak activity and duration of episodes of estrus than primiparous cows (Madureira et al., 2015a). Similarly, López-Gatius et al. (2005) found that for each additional parity number, walking activity at estrus was reduced by 21.4%. On the contrary, Walker et al. (1996) described that duration of estrus was nearly 50% shorter for primiparous than for multiparous lactating dairy cows. In addition, two other studies reported no association between parity and physical activity at estrus (VeerKamp et al., 2000; Løvendahl and Chagunda, 2010). Methodological differences may explain variation among different studies on the association between parity and physical activity, such as frequency of data transmission from sensors to software, or different breeds of cows. Moreover, the detailed information about different AAM systems reading correlations will be key to properly use automated behaviour data with physiological parameters. In a simple analysis by our group comparing a neck vs. a leg-mounted AAM, correlation between the peak intensity of estrus episodes of both systems was similar, but not at a level that justifies a seamless translation of the data from one system to the other (Madureira et al., 2015a; Silper et al., 2015c). Different AAM systems will capture different movements, and different algorithms and software filter the background data in specific manners, influencing measurements of baseline levels and relative increases in activity during estrus.

Greater milk production has been negatively correlated with estrus-related activities (Lopez et al., 2004; Rivera et al., 2010). The decrease in concentrations of estradiol, possibly caused by increased hepatic blood flow and steroid clearance, is a possible cause for decreased estrus-related activities, most notably the standing to be mounted behaviour. Madureira et al. (2015a) also found greater peak intensity and duration only in animals in the lowest quartile of milk production, but not among the other categories. Therefore, our data is in partial agreement with previous research (Lopez et al., 2004; Rivera et al., 2010). However, it seems that mounting behaviour is more affected than overall physical activity measured by AAM systems. Recent studies from our group (Silper et al., 2015a; Madureira et al., 2015a) found that heifers and cows with lower baseline levels of activity tend to have greater relative activity increase, but not necessarily greater absolute increases in step counts during estrus. In spite of the results discussed above, peak intensity during estrus was still weakly associated with milk production, emphasizing the influence of other factors such as body condition score (BCS) and parity, and probably group size, health status, and lameness (López-Gatius et al., 2005; Morris et al., 2009).

Some studies have found negative effects of milk production on conception rates (López-Gatius et al., 2005; Valenza et al., 2012), whereas others did not (Madureira et al., 2015a). The ability of individual cows to cope with high milk yield and current management practices are important in determining if a negative effect of lactation on overall fertility is more or less likely to occur. It is difficult to establish this relationship because cows with low milk production might be sick from diseases that also affect the reproductive tract, while high producing cows are often times the healthiest ones (Santos et al., 2009).

Body condition score was the major factor associated with physical activity at estrus and pregnancy per AI (P/AI) (Madureira et al., 2015a), supporting the findings by Løvendahl and Chagunda (2010), who observed that during early postpartum, low BCS had a negative correlation with estrous activity. Furthermore Aungier et al. (2012) reported that a 0.25 increase in BCS was significantly correlated with an increase in physical activity prior to ovulation. Cows that lost less than 100 kg of BW from 2 weeks pre-calving to 5 weeks post-calving had greater intensity of estrus in the first two estrus episodes post-partum (Burnett et al., 2015). The specific mechanism by which a temporary state of negative energy balance reduces estrogen-dependent estrus behaviour is unclear.

Ovarian Follicle Dynamics

Ovulation of larger follicles by lactating cows could be a result of extended follicular dominance or prolonged proestrus, which originate from lower progesterone concentrations, lower estradiol concentrations, and longer time interval for induction of GnRH and LH surges. Follicle diameter and estradiol concentration in plasma have been reported to be negatively correlated in cows (Saumande and Humblot, 2005), or to not correlate at all in heifers and cows (Aungier et al., 2015; Madureira et al., 2015a; Silper et al., 2015c). Larger follicles are more likely to fail to ovulate, and if ovulation occurs, oocytes are less likely to be fertilized. Greater incidence of ovarian abnormalities (e.g. ovulation failure, multiple ovulations, ovarian cysts) in lactating cows might originate from lower circulating estradiol in the preovulatory period of the previous estrous cycle (Sartori et al., 2004).

The correlation between the preovulatory follicle diameter and plasma estradiol is weak (Silper et al., 2015c) and is in agreement with values reported elsewhere (Sartori et al., 2004; Walker et al., 2008). Although a larger follicle is associated with greater concentration of estradiol in plasma (Cerri et al., 2004), it is clear from the current experiment that parity, BCS and ultimately milk production are the factors with the greatest impact on circulating concentrations of estradiol. Cows classified as having high activity had similar preovulatory follicle diameter, but slightly greater concentration of estradiol in plasma than cows classified as low activity (Madureira et al., 2015a). In spite of the differences in estradiol concentrations found when cows were divided in categories by estrous activity, the peak intensity measured by different AAM systems was only weakly correlated with concentration of estradiol in plasma, demonstrating a greater than expected variation. A recent study by Aungier et al. (2015) reported no correlation between activity clusters measured by AAM and follicle stimulating hormone, LH and estradiol profiles. However, a greater peak concentration of estradiol in plasma was associated with standing and estrus-related behaviours.

The ovulation of preovulatory follicles with similar diameter would suggest little change in concentrations of progesterone after AI. Data from Madureira et al. (2015) suggest that concentrations of progesterone 10 days after AI was greater in cows displaying high intensity estrus at AI. The faster increase in progesterone early in the cycle could result in increased early embryonic development (Mann and Lamming, 2001). This could represent, therefore, a possible cause for the increased P/AI found in animals with greater peak activity at estrus.

■ Detection of Estrus and Relative Intensity: Consequences to Fertility

Some estrus detection methods and aids include visual observation, tail chalk, pressure patches, pedometers and sensors. Visual observation has high labour demands and, normally, low efficiency. Therefore, TAI following hormonal manipulation of the estrous cycle has been used as an alternative for achievement of reproductive goals without the necessity of estrus detection. This implies better overall pregnancy rates because of increased rate of submission to AI. No major improvement in conception rates has been observed with TAI (Santos et al., 2009), although more recent ovulation synchronization protocols that include an intensive pre-synchronization (Double-Ovsynch) and double injections of PG before AI to ensure complete luteolysis (Wiltbank et al., 2015) result in conception rates of ~50% at the first post-partum AI.

There are plenty of AAM systems available for dairy farmers, but further exploration of the capability of different systems is necessary. Some of these systems have resources such as adaptable thresholds per farm or groups of cows, but these do not seem to be explored or extensively used. For example, adjustments could be made according to season of the year or level of milk production. These examples of possible adjustments also illustrate a challenge to the allied dairy industry related with sensors in general. There is a learning curve on how to use these systems. Even the simplest of AAM will probably require some time and patience from herd personnel in order to learn and extract the most useful information from sensors and respective software.

Detection of Estrus and Activity Monitors

Automated systems currently can be different regarding their output or variable to be analyzed (e.g. step counts, acceleration of movement, rumination time/frequency, lying time/bouts). Some examples are ALPRO (DeLaval; Sweden), SmartDairy Activity (Boumatic, USA), AfiTag (Afimilk, Israel), CowAlert (IceRobotics, UK) and Heatime HR Tag (SCR Engineers, Israel). These AAM proved to be efficient at detecting estrus. Using Heatime, Valenza et al. (2012) detected 71% of the preovulatory phases, but missed 13% of the recorded ovulations by ultrasonography. Using the same system, Aungier et al. (2012) also reported 72% of the preovulatory follicular phases identified correctly, but 32% of false-positives. It is possible that the percentage of false positives was overestimated because the cut point used to determine high progesterone status (false-positive estrus) was extremely low (progesterone > 0.6 ng/mL). Moreover, a study from Denmark (Løvendahl and Chagunda, 2010) using activity tags also showed a 74.6% estrus detection rate and 1.3% daily error rate when using the most efficient algorithm calculated by the authors. The study demonstrates the great potential of this technology to solve the estrus detection problem in commercial dairy herds.

Rumination is another parameter that can be used for automated detection of estrus. Changes in feeding behaviour, which are in accordance with increased physical activity and restlessness characteristics of estrus, result in decreased rumination time during estrus. Pahl et al. (2015) demonstrated reduction of feeding time and rumination time at day -1 and day 0 relative to AI. Reduction of time spent at each visit to the feed bunk could be another indicator of restlessness. The rumination data in the Heatime system is used in combination (not alone) to assist the activity data in detecting estrus. Probably more research is needed to validate its use as a stand-alone.

There has been little research on the use of lying and standing behaviour for estrus detection. Recently, our group has analyzed lying and standing information in relation to the estrous period in more detail (Silper et al., 2015b; Silper et al., 2017). Results from these studies indicate a large potential to improve the accuracy of estrus detection, as well as the use of quantitative information (e.g. proportional changes on lying behaviour on the day of estrus in relation to the day before and after) from these monitors to assist farm-level decision-making regarding breeding. One AAM system (AfiTag, Afimilk) uses steps, lying time and an index of restlessness in its estrus detection algorithm, but literature regarding its efficiency and measurements of estrus expression is still unclear. Given the variability reported by many and the low levels of estrus expression in general, it seems that combining measurements within one system is potentially a better alternative for reduction of false negatives. A combination of activity and lying behaviour data from IceTags (IceRobotics) significantly reduced error rate (false alerts) and increased probability of estrus detection (Jónsson et al., 2011). Peralta et al. (2005) also suggest combinations of systems are the best alternative to enhance detection and conception rates during period of heat stress. The use of more than one measurement within the same sensor can also enhance specificity and reduce false positives (Firk et al., 2002).

Expression of Estrus and Fertility: Reproduction Programs

A survey of Canadian dairy herds has shown that programs based on estrus detected by AAM result in similar reproductive performance compared to TAI (Neves and LeBlanc, 2015; Denis-Robichaud et al., 2016). A few studies, normally large surveys, have been able to draw a picture of the state of reproductive programs in North America. Caraviello et al. (2006) showed that over half of all dairy farms in USA used TAI programs. In Canada, a recent large survey indicated a strong use of TAI programs, but visual detection remains the management system mostly used by farmers (Denis-Robichaud et al., 2016). This number, however, is highly dependent on region. For example, the province of Quebec, which concentrates a large number of tie-stall farms with a small number of cows, tends to use fewer reproduction programs and other technologies.

In this survey (Denis-Robichaud et al., 2016), we reported the results from 772 survey answers, which represent 6% of the total number of dairy farms in Canada. The average herd size was 84 lactating cows (median = 60; interquartile range = 40-95 cows/herd), and herds were located in all Canadian provinces. Lactating cows were housed in tie-stall (55%) and free-stall barns (45%). Automated activity monitoring systems were used in 28% of the participating herds (4% of the tie-stall, but 59% of the free-stall herds) and were consulted for high activity alerts at least twice daily by almost all (92%) users. Interestingly, 21% of the participants never confirmed heat by visual observation before insemination, while 26% always did. Results from this survey highlight the variability in reproduction management among Canadian dairy herds. Knowledge of producers’ attitudes toward different management practices should help optimize the development and implementation of reproduction management tools.

Reproductive programs with intensive use of TAI protocols are still the gold standard regarding improvements in pregnancy rates. Recent field trials have compared different “degrees” of combination of TAI and AI upon estrus detection using AAM. Conception risk (30% vs. 31%) and days to pregnancy (137 and 122 d to pregnancy) were not different among cows bred by TAI or following estrus detection by Heatime (Neves et al., 2012). Other recent studies have experimented with different combinations of use between AAM and TAI programs (Valenza et al., 2012; Burnett et al., 2017) and overall results indicated that it is possible to achieve similar pregnancy rates in more estrus detection-intensive programs. Collectively, these large field trials aimed to modify several factors that are key to the response of the dairy’s reproduction program, particularly in the first AI. For instance, the voluntary waiting period varied from 50 to 100 DIM depending of the protocol. The use of pre-synchronization protocols that could either focus on induced estrus (PG based) or cyclicity and ovulation synchrony (GnRH based) were tested. All the studies demonstrated that the combination of methods (TAI and AAM) is perhaps the best option because it maintains high rates of conception while submitting a large number of animals to AI. In this case TAI protocols are still necessary as a safe guard for a proportion of animals that would not be bred upon estrus up to 100 DIM. The question of when to intervene with TAI protocols is probably an area that could still gain valuable information from future research. It is very likely that the adoption of AAM systems as part of a large reproduction program will vary largely from farm to farm. Work from Neves et al. (2012) and Burnett et al. (2017) demonstrated a large variation among farms in the adoption of TAI and AI upon AAM alerts within the same protocol. Another advantage of the combination of the TAI and AAM is probably the reduction in the use of pharmacological interventions. However, it is unknown how these programs would perform in areas where cows are exposed to intense heat stress, as temperature has a major impact on the detection of estrus and its intensity, which is dramatically reduced.

Expression of Estrus and Fertility: Display and Intensity near AI

In the current study, some major risk factors related with peak intensity and duration of estrus events were assessed. Even though new technologies capture physical activity using sensors and algorithms for data processing that are significantly different than those used in recent past, it was interesting to observe a lack of, or relatively weak correlation between measurements of estrus expression and milk production and preovulatory follicle diameter. Several studies using different AAM systems, farms, season and geographical location consistently observed substantial increases in P/AI from events of estrus with high peak activity (Madureira et al., 2015a; Madureira et al., 2015b; Burnett et al., 2017; Figure 1) and large decreases in lying time on the day of estrus (Silper et al., 2015d). Improvement in fertility was somewhat expected from cows with greater intensity of estrus expression; however, this was commonly associated with improvements in BCS, lower milk yield, parity and even health status. In fact, we have observed greater peak intensity and duration as BCS increased as well as in primiparous cows, but greater P/AI still occurred in spite of those and other risk factors known to affect conception rates. It is possible that information already available in herd management software used on commercial dairy farms could be used to adjust AAM systems to take into account present phenotypical conditions of the cow. The use of peak intensity and duration measurements could assist in the prediction of fertility and improve decision-making in reproductive programs using AAM. Moreover, there is potential to use AAM systems as an objective and accurate tool to select animals of superior estrus expression, although this topic still warrants further research.

Cows with high peak intensity had approximately 12 to 14 percentage units greater P/AI than cows with low peak intensity, which represents a 35% improvement in fertility (Madureira et al., 2015a;b). Previously, Lopez-Gatius et al. (2005) reported an improvement of 1.001-fold for every unit of relative increase in walking activity.

It was previously mentioned that preovulatory follicle diameter was not different between peak intensity categories, but that does not imply that proestrus or dominance length was similar as there was no control of follicular emergence in recent studies. Therefore, proestrus and dominance length (Bleach et al., 2004; Cerri et al., 2009) cannot be ruled out as possible causes related to the reduced fertility observed. Another possible factor influencing P/AI is the ovulation response from cows with different peak intensity at estrus. Madureira et al. (2015b) observed a greater failure of ovulation in cows that displayed estrus with a relative increase in peak intensity from 80 to 100%, the lowest relative increase possible after crossing the threshold from the AAM used. In a more recent study using lactating cows (Burnett et al., 2017), authors found a larger variation in ovulation times and a greater prevalence of cows ovulating before the expected ideal time after the beginning of estrus (Figure 2). While this observation is certainly important to explain our observations, it is limited to cows expressing very low peak intensity during estrus, as the threshold dividing high and low peak intensity categories was over 300% relative to the increase in the current study. One of the studies used ECP to induce estrus and ovulation, therefore bringing circulating estradiol to supra-physiological concentrations. In spite of this, the peak intensity measured by a pedometer system still significantly affected P/AI (Madureira et al., 2015b).

The display of estrus at AI has been associated with a reduction in pregnancy losses, regardless of the diameter of the preovulatory follicle (Pereira et al., 2014). Pereira et al. (2015) showed that this effect is true for both AI and embryo transfer based programs, indicating a possible major modification of the uterine environment as the cause for the improved fertility. In addition, results from Pereira et al. (2015) corroborate our data from beef cows that showed an extensive modulation of gene expression of key transcripts related with the immune system and adhesion molecules (Davoodi et al., 2016). Collectively, it seems that the expression of estrus has important positive effects in the maintenance of gestation (decrease in pregnancy losses between 32 and 60 days of gestation).

Bisinotto et al. (2015) aimed to modify concentrations of progesterone during the growth of the preovulatory follicle comparing the first with the second follicular wave. Major results described how exogenous progesterone (2 intravaginal devices) is able to “rescue” a preovulatory follicle of the first follicular wave to yield optimal fertility. An interesting finding from this study, related to estrus, is that animals that ovulated follicles from the first follicular wave growing under low concentrations of progesterone in plasma (worst possible scenario in this study), but that expressed estrus at AI, had P/AI similar to the other treatments.

A potential explanation to correlate intensity of estrus and P/AI, that has not been extensively studied, is that cows could have greater than expected individual variations in the ability to express estrogen receptors in the endometrium and, perhaps more importantly, in the hypothalamus. This would in turn generate cows that are more likely to translate circulating concentrations of estradiol into estrus-related behaviours, and later into a more adequate uterine environment for embryo development.

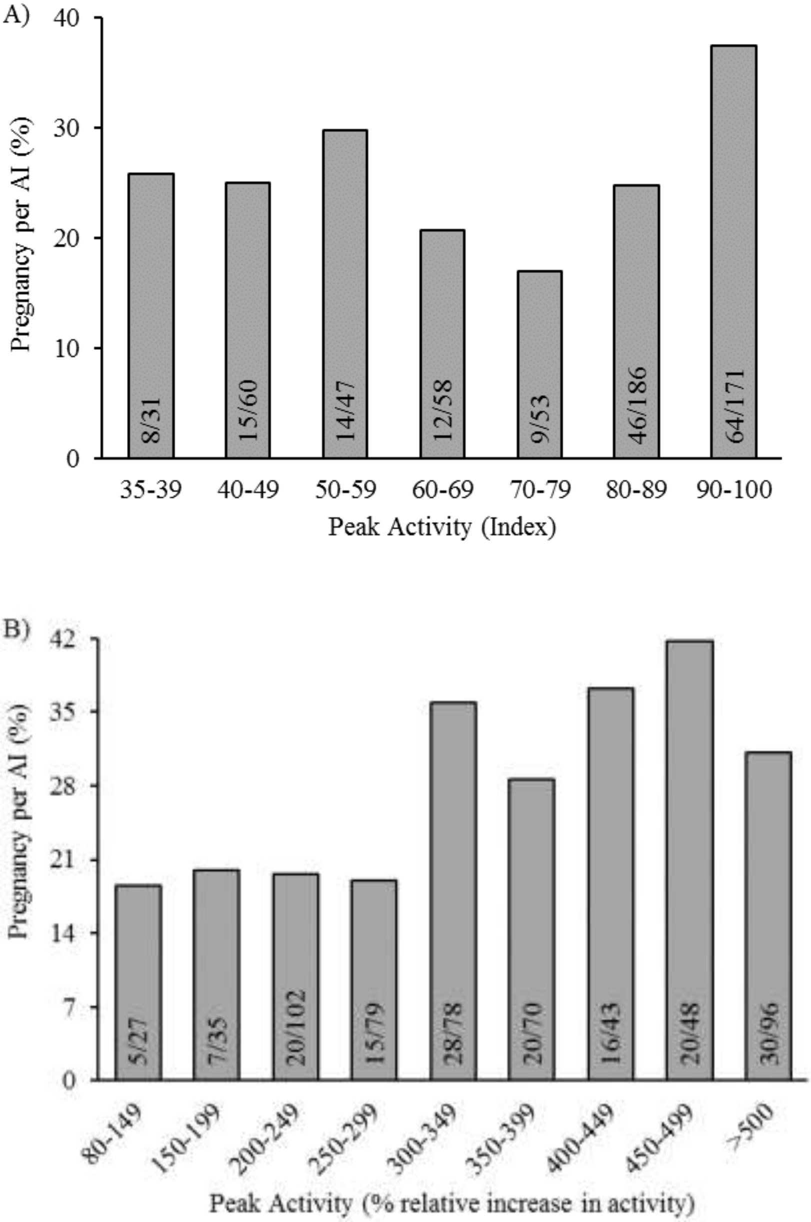

Figure 1. Distribution of pregnancy per AI (%) according to peak activity during estrus detected by A) a collar-mounted sensor and B) a leg-mounted sensor.

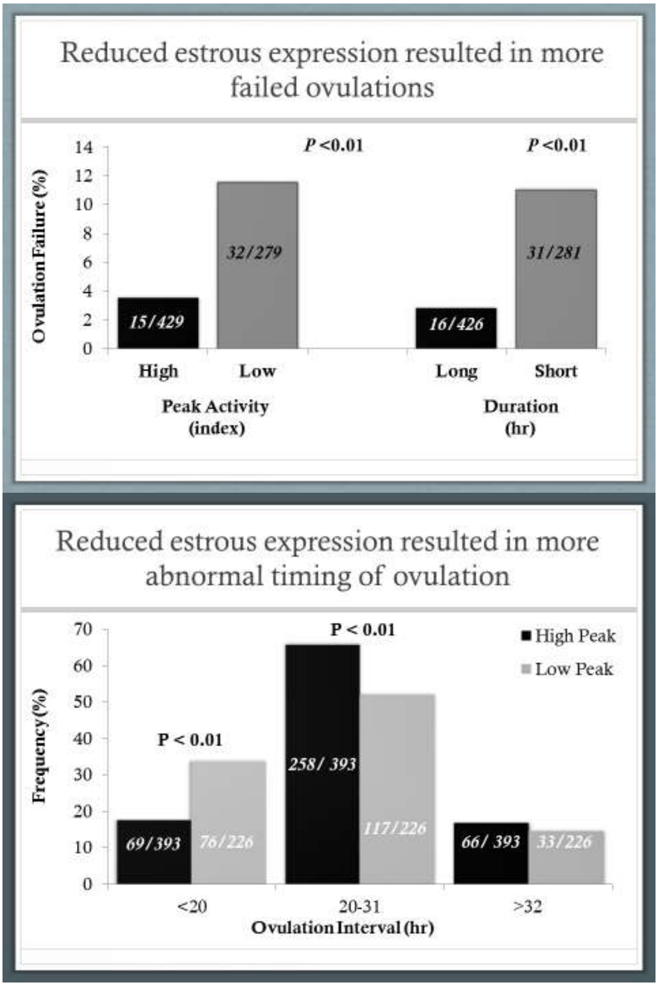

Figure 2. Ovulation failure and ovulation interval (onset of estrus to ovulation) distribution between High (≥ 300%) and Low (< 299%) relative increase at peak intensity of estrus.

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