

Using Automated Activity Monitors to Modify Reproductive Programs

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■ Take Home Messages

- More information from automated activity monitors (AAM) can be useful
 - Intensity of estrus as measured by activity monitors is closely associated with fertility. Activity monitors should be used for much more than only alerts.
 - Artificial insemination (AI) and embryo transfer (ET) can both be affected by expression of estrus and its intensity.
- Reproductive programs with strong reliance on estrus detection are highly efficient
 - Combination with timed AI is likely still necessary.
 - Expect more variability among farms.
 - An injection of GnRH at AI has significantly improved fertility, particularly those with low intensity estrus.
- Usefulness and limitations of AAM in tropical conditions
 - The use of AAM in tropical regions is not as widespread as in North America or Europe.
 - Reproductive programs in tropical conditions are still heavily affected by heat stress, and the use of more intensive programs is dependent on heat detection. Recent studies have shown promise of AAM under certain circumstances.

■ Next Steps

- Refine estrus-based reproduction programs
 - Targeted programs, GnRH timing, use of sexed semen.
- Improve knowledge on automated monitor algorithms and data collection
 - Addition of easy-to-use features on commercial software.
 - Fine tune intensity thresholds from activity monitors to better predict fertility and create management tools to improve herd reproductive efficiency.
- Genetic selection
 - Collection of digital phenotypes to use in genomic evaluation. Creation of databases.

■ Summary

The actual display of estrous behaviour and the intensity of it seem to have a profound effect on fertility (Madureira et al., 2015; Burnett et al., 2017; Silper et al., 2017; Madureira et al., 2018). Most of the data 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. Studies that modified follicular dominance length (Cerri et al., 2009), concentrations of progesterone during diestrus (Cerri et al., 2011; Bisinotto et al., 2015), proestrus length and estradiol exposure (Mussard et al., 2003; Bridges et al., 2005) and production parameters (e.g., lactation and age; Sartori et al., 2002) have described these effects on fertilization, embryo quality and uterine environment, and reduction in pregnancy losses during the late embryonic development (Ribeiro et al., 2012). However, in spite of marked effects related with the aforementioned modifications of the estrous cycle, not much emphasis was previously placed on the sole or additive effect of expression of estrus on reproductive tissues. The effect of estrus on fertility will be extensively discussed in this manuscript, but it is clear that estrus has an important 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 physiological mechanisms associated with this improvement in fertility.

In order to answer some of these questions, a series of studies using AAM (e.g., accelerometers and pedometers) was performed by our group and others. In the first stages, there was a concern to revisit some concepts of which parameters are, or are not, associated with an estrus event. Also, because of the massive use of automated activity monitors (AAM) in recent years in parts of North America and Europe, large amounts of information

around the time of estrus have become available to then correlate with actual physiological events.

This manuscript will follow a rationale that includes:

- overall association of estrus events and intensity with production parameters
- the consistent and significant effect of estrus on pregnancy per artificial insemination (P/AI) and pregnancy loss
- the possible causes for such an effect (e.g., ovulation failure, endometrium environment)
- estrus based reproductive program effectiveness and recent tools developed to improve its efficiency.

■ 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 whether 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 freestall barns and concrete flooring for an activity 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 because this data was confounded by timed AI 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.

A large field study (Lopez-Gatius et al., 2005) described that the two main factors affecting activity increase were lactation number and milk production, whereas the degree of activity increase was positively correlated with fertility after AI. The latter was not clearly stated by the author but was later corroborated by recent studies (Madureira et al., 2015). Milk production, for example, seems to affect the overall sensitivity of pedometers or activity monitors to detect true events of estrus behaviours (Holman et al., 2011). However, none of the studies above measured more detailed reproductive physiological events associated with natural estrus behaviours and the level of activity of AAM systems associated with those events. In addition, just recently more robust studies using adequate number of observations of estrus and cows have been published for more reliable conclusions.

Parity

A recent study by our group identified several risk factors associated with the intensity of estrus expression. In our study, multiparous cows expressed lower peak activity and duration of episodes of estrus than primiparous cows (Madureira et al., 2015). 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. Our study does not support findings from recent studies that reported no association between parity and physical activity at estrus (VeerKamp et al., 2000; Løvendahl and Chagunda 2010). Methodology differences may explain variation among 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 acceptable, but not at a level that justifies a seamless translation of the data from one system to the other (Madureira et al., 2015; Silper et al., 2015c). Different AAM systems will capture different movements and different algorithms and software filters the background data in specific manners, therefore influencing measurements of baseline levels and relative increases in activity during estrus.

Milk Production

Greater milk production has been negatively correlated with standing to be mounted at estrus (Lopez et al., 2004; Rivera et al., 2010). The decrease in concentrations of estradiol, possibly caused by increased hepatic blood flow and steroid clearance (Vasconcelos et al., 2003), is a possible cause for decreased estrus-related behaviour, most notably the standing to be mounted behaviour. Madureira et al. (2015) also found greater peak intensities and duration in animals with lower milk production, but the difference was most noted in the lowest quartile category. We could assume that the data partially agree with previous research (Lopez et al., 2004; Rivera et al., 2010); however, it seems that mounting activity is more affected than overall physical activity measured by AAM systems. Recent studies from our group (Silper et al., 2015a; Madureira et al., 2015) found that heifers and cows with lower baseline levels of activity tend to have greater relative activity increases, 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 (Figure 1), emphasizing the influence of factors such as body condition score (BCS) and parity, and probably other

factors such as group size, health status, and lameness (López-Gatius et al., 2005; Morris et al., 2009).

Some have found negative effects of milk production on conception rates (López-Gatius et al., 2005; Valenza et al., 2012), whereas others did not (López-Gatius et al., 2006; Madureira et al., 2015). 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 will also affect the reproductive tract, while high producing cows are often times the healthiest ones (Santos et al., 2009).

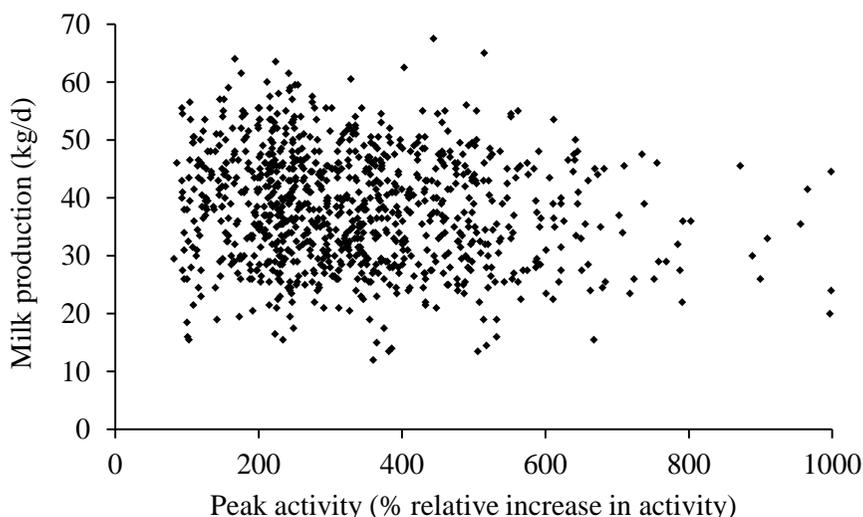


Figure 1. Correlations between milk production at the day of AI and percent relative increase in activity measured by a leg-mounted sensor ($r = 0.05$, $P < 0.01$; Madureira et al., 2015)

Body Condition Score

Body condition score was the major factor associated with physical activity at estrus and P/AI (Madureira et al., 2015). This study supported conclusions by Løvendahl and Chagunda (2010), who observed that in the first five months after calving, low, early postpartum BCS had a negative correlation with estrus activity. Additionally, 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 body weight from two weeks pre-calving to five 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.

■ **Detection of Estrus and Relative Intensity**

There are plenty of systems available for dairy farmers, but further exploration of the AAM 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, parity and BCS. These examples of possible adjustments illustrate the challenge ahead of the dairy industry and the agribusiness in general regarding the fast transformation toward heavy use of data management and automation. There is a learning curve on how to use these systems. Even the simplest AAM will probably require some time and patience from herd personnel in order to learn and extract the most from sensors and respective software.

Reproduction Programs and AAM use in North America

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 North America used timed AI programs, but at the time (mid 2000's) the use of AAM in American farms was likely very small. In Canada, a recent large survey indicated a strong use of timed AI 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, Quebec, which has many tie-stall farms with small numbers of cows, tends to use fewer AAM systems than places like British Columbia where well over half of the herds detect estrus based on AAM.

In this survey (Denis-Robichaud et al., 2016), we reported the results from 772 survey answers, which represents 6% of the total number of dairy farms in Canada. The average herd size was 84 lactating cows (median = 60) with herds located in all Canadian provinces. Lactating cows were housed in tie-stall (55%; most in Quebec) and free-stall barns (45%). AAM 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.

Automated Activity Monitors

Currently, AAM are different (e.g., step counts, acceleration of movement, rumination time/frequency, lying time/bouts) regarding their output or variable to be analyzed. Some examples are DELPRO (DeLaval; Sweden), Heatseeker II (Boumatic, USA), CowScout (GEA, Germany), AfiAct II (Afimilk, Israel), CowAlert (IceRobotics, UK) and HR Tag (SCR Engineers, Israel). These AAM are efficient at detecting estrus. Using a neck-mounted device, Valenza et al. (2012) detected 71% of the preovulatory phases, but missed 13% of the recorded ovulations. Similarly, with the same sensors, Aungier et al. (2012) reported that 72% of the preovulatory follicular phases were identified correctly, but there were 32% of false-positives. In the studies conducted by our group, the positive predictive value for estrus alerts is around 85–90%. It is possible that some of these false positives did not occur because the cut point used to determine high progesterone status (false-positive estrus) was extremely low (progesterone > 0.6 ng/mL). Progesterone in milk of 3 ng/ml or higher indicates presence of an active corpus luteum (CL). A study from Denmark (Løvendahl and Chagunda, 2010) using activity tags also showed a 74.6% detection rate and 1.3% daily error rate when using the most efficient algorithm calculated by the authors.

There has been little research on the use of lying and standing behaviour for estrus detection. Rutten et al. (2013) reviewed 48 papers but only two reported lying and standing information (De Mol et al., 2009; Brehme et al., 2008). 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 the detection of estrus, as well as the use of quantitative information (e.g., proportional changes on lying behaviours on the day of estrus in relation to the day before and after) from these monitors to assist farm-level decision-making regarding breeding. To our knowledge only one paper (Brehme et al., 2008) described the absence of lying time over long periods (16 h) during estrus. However, this paper does not provide detailed information about measurements or factors that affect lying time. One AAM system (AfiAct II, 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 periods 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

Effect of Display and Intensity of Estrus in P/AI and Pregnancy Loss

In a series of recent studies using different AAM systems, farms, timing of studies and geographical locations, substantial increases in P/AI from events of estrus of high peak activity (Madureira et al., 2015; Burnett et al., 2018; Madureira et al., 2018) and large decreases in lying time on the day of estrus (Silper et al., 2017) were consistently observed. It is commonly believed that cows that show “good” heat are more fertile; however, this tends to be associated with changes in BCS, milk yield, parity and even health status. Consistently, cows with high peak intensity had approximately 10 to 14 percentage units greater P/AI than cows with low peak intensity, which represents a 35% improvement in fertility (Figure 2; Madureira et al., 2015; Burnett et al., 2018; Madureira et al., 2018). Previously, only Lopez-Gatius et al. (2005) had reported an improvement associated with a relative increase in walking activity. It is possible that information already available in herd management software could be used to calibrate AAM 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 activity monitors. Moreover, there is potential to use AAM systems as an objective and accurate tool to select animals of superior estrus expression and fertility, although this topic still warrants further research.

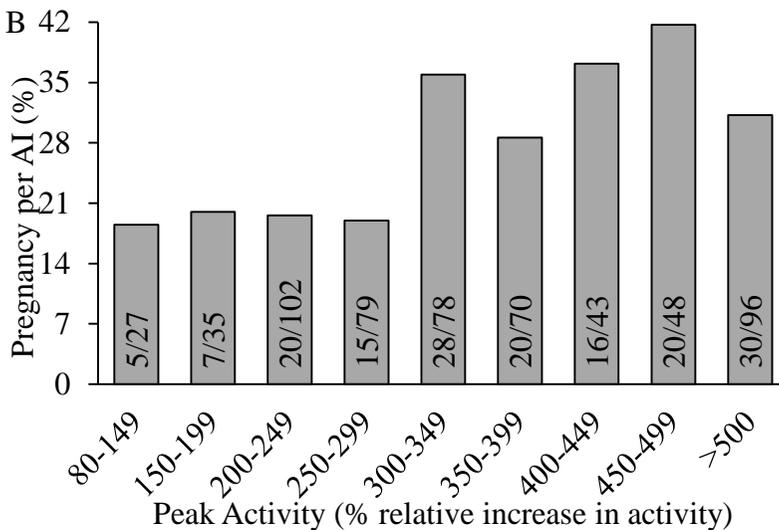


Figure 2. Distribution of pregnancy per AI (%) according to peak activity during estrus detected by a leg-mounted sensor (Madureira et al., 2015).

The display of estrus only (no distinction of intensity) at AI has been associated with a reduction in pregnancy losses (Pereira et al., 2014). Pereira et al. (2016) also performed a large field trial to describe the immense impact of estrus expression on the reduction of pregnancy losses. This study showed that this effect is true for both AI and embryo transfer (ET) based programs, indicating a possible major modification of the uterine environment as the cause for the improved fertility. Furthermore, Pereira et al. (2015) also reported that animals that display estrus at AI had decreased pregnancy losses regardless of the diameter of the pre-ovulatory follicle, which is something we normally observe in our studies regarding intensity of estrus. Most recently, another data set from Brazil (Madureira et al., 2018) also demonstrated the immense effect of estrus intensity on pregnancy loss. Cows with greater intensity of estrus had significant decreases in late embryonic/early fetal losses (Figure 3) demonstrating that the conceptus-endometrium communication in several stages of early pregnancy is compromised. This practical result from Pereira et al. (2015) and Madureira et al. (2018) corroborates 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; Povoas et al., not published). Collectively, it seems that the expression and intensity of estrus have important positive effects in the gestation maintenance, particularly by setting an endometrium environment that is more ideal to receive the conceptus.

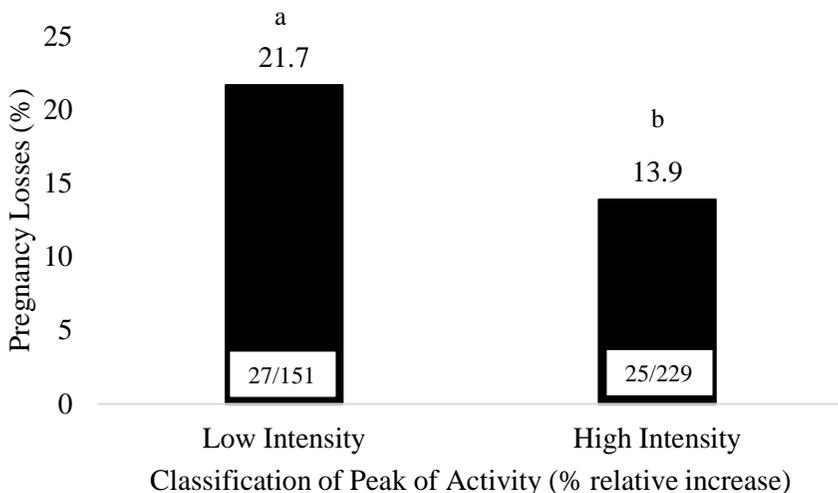


Figure 3. Pregnancy losses (%) according to categories of peak activity during estrus: Low Intensity (< 300 % relative increase) and High Intensity (\geq 300 % relative increase) at estrus episodes detected by the activity monitor (P = 0.03; Madureira et al., 2018)

Why Absence of Estrus or Low Intensity Estrus Leads to Poor Fertility?

Ovarian Follicles and Estradiol

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 because there was no control of follicular emergence in recent studies. Therefore, proestrus and dominance length (Cerri et al., 2009) cannot be ruled out as possible causes related to the reduced fertility observed. The correlation between the preovulatory follicle diameter and plasma estradiol tends to be weak (Silper et al., 2015c; $r = 0.17$) and is in agreement with values reported elsewhere (Sartori et al., 2004; Walker et al., 2008). Although reports have found that a larger follicle is associated with greater concentration of estradiol in plasma (Cerri et al., 2004), 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., 2015). In spite of these differences in estradiol concentrations the peak intensity measured by different AAM systems was only weakly correlated with concentration of estradiol in plasma, demonstrating a greater than expected variation. Aungier et al. (2015) observed no correlation between activity clusters measured by AAM and follicle stimulating hormone, luteinizing hormone 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 between high and low estrous intensities would suggest little change in concentrations of progesterone after AI, but results shown later in this manuscript suggests that concentrations of progesterone at and post-AI are more likely causes of the P/AI and pregnancy loss observations.

Ovulation Rate and Timing

Another possible factor influencing P/AI is the ovulation profile from cows with different peak intensity at estrus. In a more recent study using lactating cows Burnett et al. (2018) 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 4). It is important to note that one of the

mentioned studies used estradiol cypionate to induce estrus and ovulation, thereby bringing circulating estradiol to high concentrations. In spite of this, the peak intensity measured by a pedometer system still significantly affected P/AI results (Madureira et al., 2018).

Madureira et al. (2018) and Burnett et al. (2018) observed, using different AAM systems, a greater rate of ovulation failure in cows that displayed estrus of low intensity. In general, of the estrus episodes detected by different AAM, about 5–7% fail to ovulate. However, nearly all of that failure is associated with cows expressing low intensity estrus.

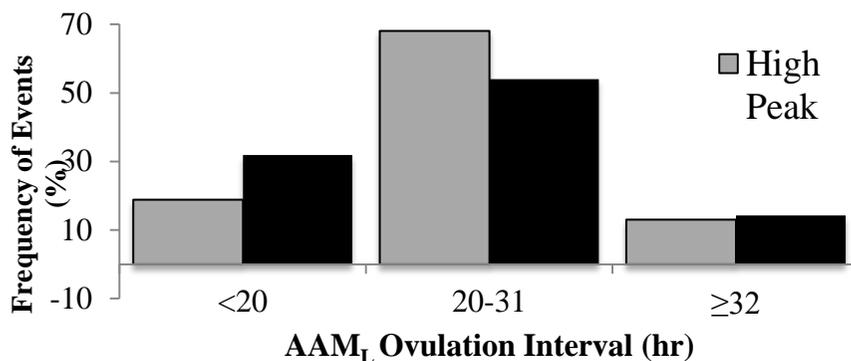


Figure 4. Frequency of estrus events for each ovulation interval with high and low estrous expression using peak activity measured by a leg-based automated activity monitor (AAM_L; $P < 0.01$; Burnett et al., 2018).

Progesterone

A study by 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 (two intravaginal devices) can “rescue” a preovulatory follicle of the first follicular wave to yield optimal fertility. 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 best treatments. A study just completed by our group (Madureira et al., unpublished) aimed to determine the impact of estrus expression detected by an AAM, on progesterone concentrations at AI (665 events) and post-AI (171 estrus events). Animals had their ovaries scanned by ultrasound at each collection for confirmation of ovarian structures. Animals with low activity had higher concentrations of progesterone and lower concentrations of estradiol upon detection compared with animals with high activity (1.0 ± 0.2 ng/ml vs. 0.3 ± 0.2 ng/ml; $P < 0.01$ and 4.6 ± 0.3 pg/ml vs. 6.7 ± 0.2 pg/ml; $P < 0.01$). Follicle diameter did not differ between animals with high or low peak of activity ($P = 0.41$), but much higher

concentrations of progesterone on days 7, 14 and 21 post-AI were found in animals with greater estrus expression (7 days – High: 3.4 ± 0.2 pg/ml vs. Low: 2.7 ± 0.2 pg/ml; $P < 0.05$; 14 days – High: 4.9 ± 0.4 pg/ml vs. Low: 2.9 ± 0.4 pg/ml; $P < 0.01$ and 21 days – High: 6.8 ± 0.3 pg/ml vs. Low: 5.4 ± 0.3 pg/ml; $P < 0.01$). Size of the CL on days 7, 14 and 21 post-AI did not differ between animals that expressed high or low activity. In conclusion, animals that had higher expression of estrus had greater P/AI and a progesterone profile at and post-AI normally associated with improved early embryonic development.

Endometrium Environment

Several studies have shown the dominant effect of pre- and post-exposure of progesterone relative to AI, proestrus length or estradiol levels on reproductive tissues, particularly the endometrium and the conceptus at various stages of early development. Studies that modified follicular dominance length (Cerri et al., 2009), concentrations of progesterone during diestrus (Cerri et al., 2011), proestrus length and estradiol exposure (Mussard et al., 2003; Bridges et al., 2005), production parameters (e.g., lactation and age; Sartori et al., 2002) and most recently health (Ribeiro et al., 2016) have described these effects on fertilization, embryo quality and uterine environment. However, in spite of marked effects related with the aforementioned modifications of the estrous cycle, not much emphasis has been placed on the isolated or additive effect of expression of estrus on reproductive tissues. In order to answer some of these questions, we investigated the association of estrus expression at the time of AI with the expression of critical genes in the endometrium, CL and embryo during the pre-implantation period, specifically on day 19 of gestation (Davoodi et al., 2016). In addition, the difference in estrus expression was evaluated 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 estrus 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 PG synthesis (Figure 5). 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 favours CL maintenance and secretion of progesterone, both key to sustaining pregnancy (Davoodi et al., 2016). Moreover, cows that displayed estrus yielded longer conceptuses, which can be associated with better chances of survival. The effects of expression of estrus seems to interact with progesterone concentration on day 7 of the estrous cycle in a way that

positively influences endometrium receptivity and embryo development. However, the specific causes that lead to the presence or absence of estrus expression are unknown (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.

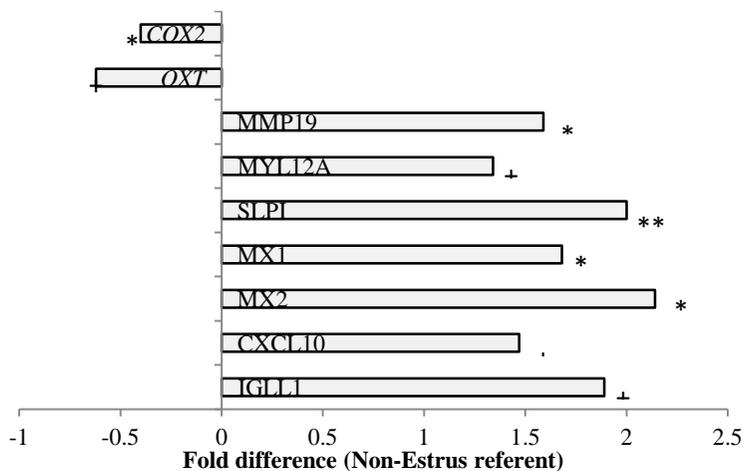


Figure 5. Effect of estrus expression on endometrium gene expression. Significant fold difference based on non-estrus expression as referent has been shown for genes with a significant pattern of expression in endometrium tissue (Davoodi et al., 2016).

An even more recent study (Povoas et al., unpublished) followed a similar experimental design from the previous study, but now using a different day of gestation (day 15). The results also showed a similar trend toward a more developed conceptus, almost twice the length compared with those coming from cows that did not display estrus. Most importantly, the transcriptome of the endometrium on day 15 of gestation was differentially expressed (Figure 6). No genes related to morphogenesis or maternal recognition of pregnancy were differentially expressed among cows that expressed or did not express estrus. These results evidence differential gene expression on day 15 endometrium of pregnant cows according to estrus expression at timed-AI. A more favorable uterine environment in association with estrus was indeed evidenced by a significantly greater conceptus length.

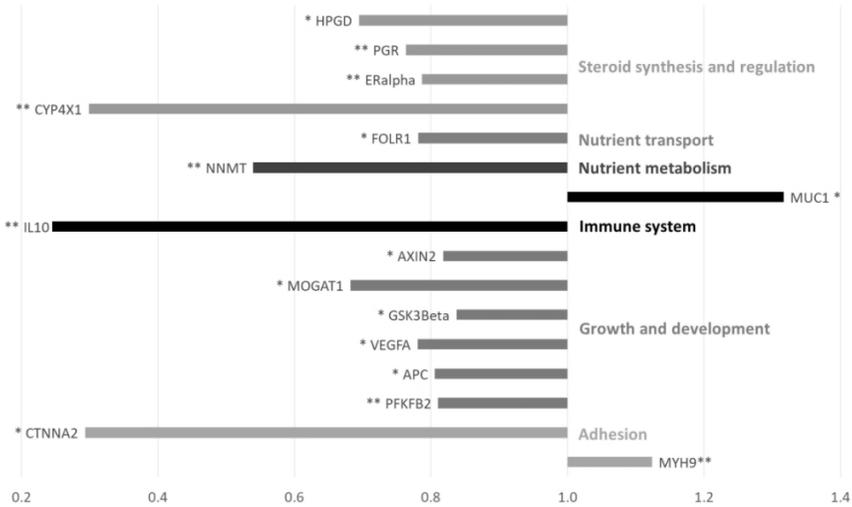


Figure 6. Effect of estrus expression on endometrium gene expression. Significant fold difference based on non-estrus expression as referent has been shown for genes with a significant pattern of expression in endometrium tissue (Povoas et al., unpublished).

Reproduction Programs

Reproductive programs with intensive use of timed AI protocols are still the gold standard as the “go to” method to improve pregnancy rates. Recent field trials have compared different degrees of combination of timed AI and AI upon estrus detection using AAM. Conception risk (30% vs. 31%) and days to pregnancy (137 and 122) were not different among cows bred by timed AI or following estrus detection by an AAM system (Neves et al., 2012). Other studies have experimented with different combinations of use between AAM and timed AI programs (Valenza et al., 2012; Stevenson et al., 2014; Fricke et al., 2014; Burnett et al., 2017; Denis-Robichaud et al., 2018). Overall results indicated that it is possible to achieve similar pregnancy rates in more estrous 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 days in milk (DIM) depending of the treatment. 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 (timed AI and AAM) is perhaps the best reproduction program because it maintains high rates of conception while submitting large numbers of animals to AI, while reducing to a certain extent the use of pharmacological assistance. It is fair to say timed AI 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 timed AI protocols is probably an area that could still gain valuable information from future research. The example of the work performed in Ontario (Denis-Robichaud et al., 2018) is probably the most extreme when comparing a TAI only program vs. one that allows long periods for spontaneous estrus detection after the end of the voluntary waiting period. In summary, there will be several factors that will influence the final result of the reproductive program at specific farms, but the literature now suggests that AAM can be incorporated into it without loss of efficiency. A very recent study performed in British Columbia (Burnett et al., unpublished) tested whether it was possible to use the information from the AAM to modify a breeding decision at the farm level. Animals were divided into four groups based on the intensity of estrus expression and on GnRH treatment at the time of AI: 1) high estrus expression with no GnRH injection (HighNG), 2) low estrus expression with no GnRH injections (LowNG), 3) high estrus expression with a GnRH injection (HighG), and 4) low estrus expression with a GnRH injection (LowG). The hypothesis was that, based on the previous results showing unfavourable ovulation failure rate and timing in cows expressing low intensity estrus, the LowG group would significantly improve P/AI. The study has not been completed just yet but the results so far are positive. The LowG group not only improved P/AI, but did so up to levels similar found in the high intensity estrus groups (Figure 7).

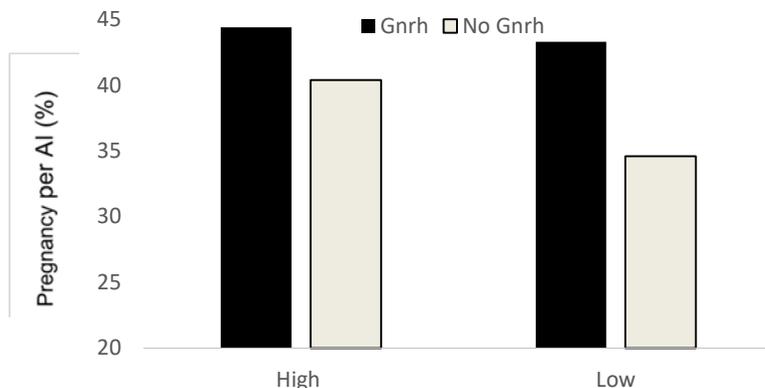


Figure 7. Pregnancy per AI (%) according to categories of peak activity during estrus: Low Intensity vs. High Intensity (threshold of approximately 250% relative increase) (Burnett et al., unpublished).

Lastly, our group tested the effect of estrus intensity on success of embryo transfer (ET) and collection (Burnett et al., unpublished). In the first experiment, Holstein heifers (10.5 to 14.5 months) were superovulated (n = 69 from 51 animals) for the collection of embryos, and on the day of estrus, the total number of follicles were counted. Then, embryos were collected, counted and assessed for viability. In Experiment 2, Holstein cows were

synchronized and at 7 days post-estrus those bearing a CL were implanted with an embryo ($n=1,147$ from 657 cows). Overall, cows with higher peak activity had a higher number of total embryos collected (10.2 ± 1.2 vs. 6.0 ± 1.3 embryos; $P = 0.01$) and a higher percent of those embryos were viable (53.1 ± 5.0 vs. $23.4 \pm 5.1\%$; $P < 0.001$). In the second experiment, 89% of cows expressed estrus prior to ET. Animals expressing estrus before ET had substantially higher P/ET than those that did not (35.8 ± 1.6 vs. $5.9 \pm 4.9\%$; $P < 0.001$). Of the animals that expressed estrus, cows with higher estrus expression had higher ET success than those with low estrus expression (41.5 ± 2.3 vs. $30.6 \pm 2.2\%$; $P < 0.001$). In conclusion, estrus expression is important for both periods before and after ET as seen by more viable embryos and higher P/ET for animals with greater estrus expression.

It is very likely that the adoption of AAM systems as part of a large reproduction program will vary largely from farm to farm. There is a large variation by farm in the adoption of timed AI and AI upon AAM alerts within the same treatment (Neves et al., 2012; Burnett et al., 2017; Denis-Robichaud et al., 2018). Another possible advantage of the combination of timed AI and AAM is the reduction in the use of pharmacological interventions. However, we don't know how these programs would behave under sites exposed to intense heat stress such as Brazil, as temperature tends to have a major impact on the detection of estrus and intensity.

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