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**TITLE:** Behavioral and physiological changes around estrus events identified using multiple automated monitoring technologies

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1   **Interpretive Summary:** *Behavioral and physiological changes around estrus events identified*  
2   *using multiple automated monitoring technologies. Dolecheck.* The objectives of this study were  
3   to describe estrus-related changes in multiple parameters collected by automated technologies and  
4   to explore the application of machine learning techniques to automatically collected data. Activity  
5   level, lying bouts, lying time, rumination time, feeding time, and reticulorumen temperature  
6   showed differences between periods of estrus and non-estrus, but ear surface temperature did not.  
7   Additionally, applying machine learning techniques to automatically collected technology data  
8   shows potential for estrus detection.

9

## 10   TECHNOLOGY USE FOR ESTRUS DETECTION

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12   **Behavioral and physiological changes around estrus events identified using multiple**  
13   **automated monitoring technologies**

14

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22     **ABSTRACT**

23         This study included two objectives. The first objective was to describe estrus-related  
24     changes in parameters automatically recorded by the CowManager SensOor (Agis  
25     Automatisering, Harmelen, Netherlands), DVM bolus (DVM Systems, LLC, Greeley, CO), HR  
26     Tag (SCR Engineers Ltd., Netanya, Israel), IceQube (IceRobotics Ltd., Edinburgh, Scotland), and  
27     Track a Cow (Animart Inc., Beaver Dam, WI). This objective was accomplished using 35 cows  
28     in 3 groups between January and June 2013 at the University of Kentucky Coldstream Dairy. A  
29     modified Ovsynch with G7G protocol was used to partially synchronize ovulation, ending after  
30     the last PGF<sub>2α</sub> injection (day 0) to allow estrus expression. Visual observation for standing estrus  
31     was conducted for 4, 30-min periods at 0330, 1000, 1430, and 2200 on days 2, 3, 4, and 5.

32         Eighteen of the 35 cows stood to be mounted at least once during the observation period.  
33     These cows were used to compare differences between the 6 h before and after the first standing  
34     event (estrus) and the two weeks preceding that period (non-estrus) for all technology parameters.  
35     Differences between estrus and non-estrus were observed for CowManager SensOor minutes  
36     feeding per h, minutes of high ear activity per h, and minutes ruminating per h; twice daily DVM  
37     bolus reticulorumen temperature; HR Tag neck activity per 2 h and minutes ruminating per 2 h;  
38     IceQube lying bouts per h, minutes lying per h, and number of steps per h; and Track a Cow leg  
39     activity per h and minutes lying per h. No difference between estrus and non-estrus was observed  
40     for CowManager SensOor ear surface temperature per h.

41         The second objective of this study was to explore the estrus detection potential of machine learning  
42     techniques using automatically collected data. Three machine learning techniques (random forest,  
43     linear discriminant analysis, and neural network) were applied to automatically collected  
44     parameter data from the 18 cows observed in standing estrus. Machine learning accuracy for all

45 technologies ranged from 91.0% to 100.0%. When visual observation was compared to  
46 progesterone profiles of all 32 cows, a 65.6% accuracy was found. Based on these results, machine  
47 learning techniques have potential to be applied to automatically collected technology data for  
48 estrus detection.

49 **Key Words:** precision dairy farming technology, estrus detection, automated estrus detection,  
50 technology, machine learning

## 51 INTRODUCTION

52 Detecting a high percentage of cows in estrus is essential to maintain reproductive  
53 performance in dairy herds using artificial insemination. The most common form of estrus  
54 detection is visual observation, used by 93% of US dairy operations (USDA, 2007). The Dairy  
55 Records Management Systems reported mean yearly estrus detection rate on US Holstein herds  
56 (including all reproductive management strategies) as 44.9% in 2015 (DRMS, 2015). This low  
57 estrus detection rate may be a result of the extreme decline in Holstein cattle estrus duration (from  
58 18 h to less than 8 h) over the last 50 years (Reames et al., 2011). Increasing age, milk production,  
59 and environmental factors (greater ambient temperature, uncomfortable housing, etc.) can also  
60 negatively affect length and intensity of estrus expression (Vailes and Britt, 1990; López-Gatius  
61 et al., 2005; Palmer et al., 2010).

62 Automated estrus detection (**AED**) technologies are an available alternative to supplement  
63 or replace visual estrus detection. Parameters with potential for AED include mounting events,  
64 activity level, lying time, rumination events, blood or milk progesterone (**P4**) levels, feeding time,  
65 body temperature, and more (Senger, 1994; Saint-Dizier and Chastant-Maillard, 2012; Fricke et  
66 al., 2014). Estrus-related changes in some of these parameters (mounting events, activity level,  
67 lying time, rumination events, and P4) have been quantified repeatedly. However, a lack of

68 consistent data exists surrounding estrus-related changes in feeding time and body temperature.  
69 Additionally, not all of these parameters have been measured on the same cows during the same  
70 estrus periods.

71 To determine the accuracy of a specific AED technology, estrus events identified by the  
72 technology algorithm (a set of criteria used to determine “estrus”) are compared to a gold standard  
73 such as visual observation, ultrasonography, blood or milk P4 levels, or a combination of these.  
74 Correctly identified estrus events are considered true positives (**TP**), non-alerted estrus events are  
75 false negatives (**FN**), non-alerted non-estrus events are true negatives (**TN**), and alerted non-estrus  
76 events are false positives (**FP**; Firk et al., 2002). Detecting estrus events is a balance of sensitivity  
77 and specificity. Sensitivity, the probability that an event is alerted, is equal to  $TP/(TP+FN)*100$   
78 (Hogeveen et al., 2010). Specificity, the probability that when an event does not occur no alert is  
79 generated, is equal to  $TN/(TN+FP)*100$ . Because neither sensitivity nor specificity account for  
80 the prevalence of the event, other comparative measurements are also useful, including accuracy  
81  $[(TP+TN)/(TP+TN+FP+FN)*100]$ .

82 The estrus detection accuracy of a technology depends on 3 factors: 1) how strongly and  
83 discretely the measured parameters are associated with estrus, 2) how accurately the technology is  
84 measuring those parameters, and 3) if the technology manufacturer algorithm is accurately  
85 processing the data to create useful “estrus alerts.” Most technology manufacturer algorithms are  
86 proprietary, making it difficult to identify how well each of the 3 factors described above are  
87 performing. Machine learning techniques can replace the manufacturer alert algorithms and  
88 evaluate technologies based solely on parameter data collected. Mitchell et al. (1996) and Krieter  
89 (2005) have previously described the use of machine learning techniques for estrus detection.  
90 However, both studies focused on identifying the day of estrus rather than a more specific time

91 period. Additionally, no commercially available AED technologies were evaluated in those  
92 analyses.

93 This study included two objectives. The first objective was to describe estrus-related  
94 changes in neck activity, ear activity, leg activity, step count, lying bouts, lying time, rumination,  
95 feeding time, reticulorumen temperature, and ear surface temperature as measured using 5 AED  
96 technologies on the same cows. The second objective of this study was to explore the estrus  
97 detection potential of machine learning techniques using parameters collected by AED  
98 technologies.

99 **MATERIALS AND METHODS**

100 This study was conducted at the University of Kentucky Coldstream Dairy under  
101 Institutional Animal Care and Use Committee protocol number 2013-1069. All lactating cows ( $n$   
102 = 82) were housed in two groups, separated by a shared, raised feedbunk. Both groups maintained  
103 open access to freestalls, one group with sawdust-covered rubber-filled mattresses (PastureMat;  
104 Promat, Ontario, Canada) and the other group with sawdust-covered Dual Chamber Cow  
105 Waterbeds (Advanced Comfort Technology, Inc., Reedburg, WI). Cows received access to a grass  
106 seeded exercise lot for 1 h per d at 1000, weather permitting. All other surfaces accessible to cows  
107 (freestall area, feed bunk, holding pen, and alleys) contained grooved concrete. Delivery of a TMR  
108 ration containing corn silage, alfalfa silage, whole cottonseed, and grain mix occurred 2X at 0530  
109 and 1330. Milking occurred 2X at 0430 and 1530.

110 This study enrolled 32 Holstein cows that had not been bred in their current lactation.  
111 Parity, DIM at the beginning of the study protocol, and summit milk production from the current  
112 lactation of these cows was (mean  $\pm$  SD)  $2.0 \pm 1.2$ ,  $77.8 \pm 20.5$  d, and  $39.8 \pm 8.8$  kg, respectively.  
113 Cow ovulations were synchronized in three groups of 14, 10, and 8 cows, starting on January 24,

114 March 19, and May 14, respectively. The synchronization protocol (Figure 1) was a modification  
115 of the standard Ovsynch (Pursley et al., 1995), preceded by G7G (Bello et al., 2006). In contrast  
116 to the standard Ovsynch, the last injection of GnRH (gonadorelin diacetate tetrahydrate,  
117 Cystorelin; Merial Limited, Duluth, GA; 100 µg intramuscular) was not administered to stimulate  
118 estrus expression. Additionally, to stimulate corpus luteum regression, two PGF<sub>2α</sub> injections  
119 (dinoprost tromethamine, Lutalyse; Zoetis, Florham Park, NJ; 25 mg intramuscular) were given  
120 on the last day of the protocol (7 d after the first GnRH injection), 6 h apart (0800 and 1400). Day  
121 0 was designated as the last day of the synchronization protocol in each group (Figure 1).

122 ***Estrus Confirmation***

123 Visual observation of cows for 4, 30-min periods at 0330, 1000, 1430, and 2200 occurred  
124 on d 2, 3, 4, and 5 (Figure 1). Two observers were present at each shift, with one assigned to each  
125 side of the separated housing area. Study cows were clearly identified using spray paint.  
126 Observers recorded the time of each standing estrus event.

127 Blood samples (10 ml) were collected from cow coccygeal veins on d -2, -1, 0, 1, 2, 7, 9,  
128 and 11 (Figure 1). Plasma was separated from centrifuged samples and stored at -20 °C until the  
129 concentration of P4 was determined by radioimmunoassay (Coat-a-Count Progesterone, Siemens  
130 Medical Solutions USA, Inc., Malvern, PA). Response to the synchronization protocol was  
131 confirmed if P4 was greater than 1.0 ng/ml on d -2, -1, and 0, dropped to less than 1.0 ng/ml by d  
132 1, and returned above 1.0 ng/ml by d 9. The P4 results were used to determine sensitivity,  
133 specificity, and accuracy of visual observation. Only validated standing estrus events were used  
134 to describe estrus-related changes in AED parameters and to explore estrus detection potential of  
135 machine learning techniques.

136     ***Technologies Evaluated***

137         Each cow was fitted with 5 automated monitoring technologies before beginning  
138         synchronization. The CowManager SensOor (Agis Automatisering, Harmelen, Netherlands),  
139         attached to the left ear, used a 3-axis accelerometer to classify each minute into one of six behaviors  
140         (rumination, feeding, resting, low activity, regular activity, or high activity) and reported hourly  
141         percentage of time associated with each behavior. Additionally, the CowManager SensOor used  
142         a digital surface temperature monitor to evaluate mean hourly ear surface temperature. The  
143         behavioral portion of the CowManager SensOor, but not the temperature monitor, was previously  
144         validated on dairy cows (Bikker et al., 2014). The DVM bolus (DVM Systems, LLC, Greeley,  
145         CO), placed into the reticulorumen using a bolus gun, recorded reticulorumen temperature twice  
146         daily using a passive radio-frequency identification transponder. Data download occurred at the  
147         time of parlor entrance, where panel readers were located. The HR Tag (SCR Engineers Ltd.,  
148         Netanya, Israel), held on the left side of the neck using a nylon collar, measured neck activity and  
149         rumination time in 2 h blocks using a 3-axis accelerometer and a microphone containing a  
150         microprocessor, respectively. The rumination portion of the HR Tag was previously validated on  
151         dairy cattle (Schirrmann et al., 2009; Burfeind et al., 2011). The IceQube (IceRobotics Ltd.,  
152         Edinburgh, Scotland), attached to the left rear leg using a plastic strap, reported number of steps,  
153         lying bouts, and lying time every 15 minutes using a 3-axis accelerometer. The Track a Cow  
154         (Animart Inc., Beaver Dam, WI), attached to the front right leg using a nylon strap, used a 3-axis  
155         accelerometer to measure hourly activity and lying time.

156     ***Statistical Analysis***

157         All technology parameter data was averaged by 12 hour blocks of time. The 12 hour block  
158         of time used to define estrus depended on the analysis.

159        ***Estrus vs. non-estrus.*** For this analysis, if a cow was observed in standing estrus during  
160 visual observation periods (0330, 1000, 1430, or 2200), a cow's estrus was classified as starting 6  
161 h before the first observed standing estrus event and ending 6 h after the first observed standing  
162 estrus event. For example, a cow first observed in standing estrus during the 1430 observation  
163 period would have estrus defined as 0830 to 2030 of that day. The 28, 12 h periods (14 d) before  
164 the estrus period were classified as periods of non-estrus. The MIXED procedure of SAS 9.3 (SAS  
165 Institute, Inc., Cary, NC) was used to analyze the main effects of estrus status (estrus or non-  
166 estrus), parity, DIM at the start of the synchronization protocol, summit milk production, and the  
167 interaction of estrus status and selected covariates (parity, DIM at the start of the synchronization  
168 protocol, and summit milk production) on all technology parameter data, considering cow as a  
169 random effect and time as a repeated measure. All main effects were kept in each model regardless  
170 of significance level. Stepwise backward elimination was used to remove non-significant  
171 interactions ( $P \geq 0.05$ ).

172        ***Machine learning.*** For this analysis, if a cow was observed in standing estrus during visual  
173 observation periods (0330, 1000, 1430, or 2200), a cow's estrus was classified as the 12 h period  
174 of time leading up to the first observed standing estrus event. For example, a cow first observed  
175 in standing estrus during the 1430 observation period would have estrus defined as 0230 to 1430  
176 of that day. This was different from the estrus vs. non-estrus analysis because it would not be  
177 valuable for machine learning to detect estrus after the observation of standing estrus. The 28, 12  
178 h periods (14 d) before the estrus period were classified as periods of non-estrus.

179        Unmodified data, as recorded by 4 of the technologies (CowManager SensOor, HR Tag,  
180 IceQube, and Track a Cow), were used for machine learning analysis. The DVM bolus was left  
181 out of this analysis because machine learning techniques work by finding patterns between

182 parameters and are not meant to be applied to single parameter data sets. The caret package from  
183 R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to create a 4-  
184 fold cross-validation, including 10 analysis per series, using 70% of all technology parameter data.  
185 Three machine learning techniques were tested: random forest, linear discriminant analysis, and  
186 neural network. The goal of the algorithm development was to predict which time block (of the  
187 29, 12 h periods defined earlier) each data line referenced. After algorithm development, the  
188 remaining 30% of all technology parameter data was used to test prediction ability. Sensitivity,  
189 specificity, and accuracy of each technology and machine learning technique combination were  
190 calculated relative to observed standing estrus. The “exact” method was used to calculate 95%  
191 confidence intervals for each measurement (Clopper and Pearson, 1934).

## 192 **RESULTS AND DISCUSSION**

193 Progesterone analysis indicated that 29 of the 32 cows (90.6%) ovulated after completing  
194 the synchronization protocol. Eighteen cows (62.1%) were observed standing to be mounted  
195 during the visual observation periods. Failure to detect the remaining 11 cows may have resulted  
196 from unexpressed estrus or short estrus lengths that were unobserved because of non-continuous  
197 observation.

198 A researcher error resulted in some data not being properly saved from the computer.  
199 Consequently, 4 cows observed in estrus were missing lying time data as measured by Track a  
200 Cow and were removed from affected statistical analysis. Additionally, a technology malfunction  
201 resulted in no data measured by the IceQube for 1 other cow, which was also removed from  
202 affected statistical analysis. Remaining technology parameter statistical analysis included all 18  
203 cows observed in standing estrus.

204        ***Estrus vs. Non-estrus***

205            ***Activity.*** All activity measures increased during estrus compared to non-estrus (Table 1).  
206        The percent activity change between non-estrus and estrus for high ear activity as measured by  
207        CowManager SensOor, neck activity as measured by HR Tag, number of steps as measured by  
208        IceQube, and leg activity as measured by Track a Cow was 309.4%, 118.5%, 280.4%, and 237.4%,  
209        respectively (Table 1). The range of increase in activity may have resulted from differing  
210        accelerometer attachment locations. Overall, similar estrus associated increases in numbers of  
211        steps (2 to 4 times) have been reported previously (Kiddy, 1977; Redden et al., 1993; Roelofs et  
212        al., 2005a).

213            The interaction of DIM at the start of synchronization and estrus status significantly  
214        influenced all measures of activity (Table 2). Cows that started the synchronization protocol at a  
215        later DIM displayed greater estrus-related activity levels than cows starting the synchronization  
216        protocol at earlier DIM. Additionally, the interaction of parity and estrus status significantly  
217        influenced activity as measured by the IceQube and Track a Cow (Table 2). In both cases, as  
218        parity increased, estrus-related activity decreased. In agreement, López-Gatius et al. (2005) found  
219        that with each additional parity, walking activity decreased 21.4%. Other studies have identified  
220        a similar relationship (Roelofs et al., 2005a; Yaniz et al., 2006). In this study, parity only  
221        influenced estrus-related activity levels when monitored using leg mounted technologies,  
222        indicating that later parity cows increase head and neck movements during estrus, but do not walk  
223        around as much as younger cows. Activity as measured by Track a Cow was also significantly  
224        influenced by the interaction of summit milk production and estrus status (Table 2). As summit  
225        milk production increased, estrus-related activity increases were suppressed. The relationship  
226        between greater milk production and decreased estrus-related activity has previously been

227 established (López-Gatius et al., 2005; Yaniz et al., 2006; Reith et al., 2014). Why this effect was  
228 not observed by all activity measurement devices is unclear.

229         *Lying time and lying bouts.* All lying measures decreased during estrus compared to non-  
230 estrus (Table 1). The percent change between non-estrus and estrus for lying bouts as measured  
231 by IceQube, lying time as measured by IceQube, and lying time as measured by Track a Cow were  
232 similar at -51.4%, -58.9%, and -63.9%, respectively. Time spent lying decreases around estrus  
233 because of increased activity and restlessness (Esslemont and Bryant, 1976; Livshin et al., 2005;  
234 Jonsson et al., 2011).

235         The interaction of DIM at the start of synchronization and estrus status significantly  
236 influenced lying bouts as measured by IceQube and lying time as measured by Track a Cow (Table  
237 2). Cows that started the synchronization protocol at a later DIM expressed shorter lying time as  
238 measured by Track a Cow and fewer lying bouts as measured by IceQube during estrus than cows  
239 starting the synchronization protocol at earlier DIM. Why lying time as measured by IceQube was  
240 not effected in the same way is unclear. No measures of lying activity were significantly  
241 influenced by the interactions of parity or summit milk production with estrus status.

242         *Rumination and feeding time.* Both measures of rumination time decreased during estrus  
243 compared to non-estrus (Table 1). The percent change in rumination time between non-estrus and  
244 estrus for the CowManager SensOor and the HR Tag were -43.8% and -37.9%, respectively. Reith  
245 and Hoy (2012) evaluated 265 estrus events, finding that rumination on the day of estrus decreased  
246 17% (74 min), but with large variation between herds (14 to 24%). In a follow-up study that  
247 looked at 453 estrous cycles, rumination time decreased 19.6% (83 min) on the day of estrus (Reith  
248 et al., 2014). Pahl et al. (2015) also found a decrease in rumination on the day of (19.3%) and the  
249 day before (19.8%) inseminations leading to pregnancy. The comparatively large decreases in

250 rumination around estrus found in the current study could be the result of a narrower “estrus”  
251 window (12 h) as compared to the previous studies (1 d).

252 Differences between technology measured rumination times (2.66 min/h during estrus and  
253 6.48 min/h during non-estrus) could be the result of differing recording methods. The  
254 CowManager SensOor used an accelerometer to identify ear movement associated with  
255 rumination. The HR Tag used a microphone system that rested on the cow’s neck to identify the  
256 regurgitation and re-chewing of cud. Both systems have been validated with high correlations to  
257 visual observation (CowManager SensOor:  $r = 0.93$  and HR Tag:  $r = 0.93$ ; Bikker et al., 2014 and  
258 Schirmann et al., 2009). However, the CowManager SensOor validation was conducted on a per  
259 minute basis whereas the HR Tag validation was conducted on a 2-hour basis, meaning results are  
260 not directly comparable.

261 One explanation for decreased rumination around estrus is decreased feed intake (Maltz et  
262 al., 1997; Diskin and Sreenan, 2000). Conversely, feeding time as measured by the CowManager  
263 SensOor in this study increased by 8.00 min/h during estrus compared to non-estrus (Table 1).  
264 Other researchers agree that feeding behavior may not always decrease around estrus. De Silva et  
265 al. (1981) found no change in feed intake during the 3 d period surrounding estrus and Lukas et al.  
266 (2008) found DMI increased 0.61 kg/d during estrus. The method by which the CowManager  
267 SensOor measured feeding time in the current study depended on the ability of an accelerometer  
268 to distinguish ear movements related to feeding and is not a true measure of intake. Therefore, the  
269 reported increase in feeding time may not represent an actual increase in DMI, but rather an  
270 increase in head movements similar to those occurring when a cow is eating.

271 Feeding time was not significantly influenced by the interaction of DIM at the start of  
272 synchronization, parity, or summit milk production with estrus status. The interaction of DIM at

273 the start of synchronization and estrus status significantly influenced both measures of rumination  
274 (Table 2). Cows that started the synchronization protocol at a later DIM expressed a larger  
275 decrease in rumination during estrus than cows starting the synchronization protocol at earlier  
276 DIM. This result is consistent with the other observations of estrus expression in this study  
277 (activity and lying time) as DIM at the start of synchronization increased. Neither measure of  
278 rumination was significantly influenced by the interactions of parity or summit milk production  
279 with estrus status.

280         **Temperature.** Reticulorumen temperature as measured by the DVM bolus increased 0.43  
281 °C during estrus ( $P < 0.01$ ; Table 1). Ear surface temperature as measured by the CowManager  
282 SensOor increased 1.20 °C during estrus ( $P = 0.20$ ; Table 1). Although the numeric increase in  
283 ear surface temperature during estrus was greater than that of the reticulorumen temperature, it  
284 also displayed a larger variation as evident in the greater standard error (Table 1). Ear surface  
285 temperature is influenced by both core body temperature and ambient temperatures (Mader and  
286 Kreikemeier, 2006). Therefore, ear surface temperature was expected to be less than and fluctuate  
287 more than reticulorumen temperature (a measure of core body temperature alone). CowManager  
288 SensOor temperature measurements are not marketed for estrus detection use, likely because of  
289 this variation.

290         The temperature increases observed in this study (0.51 to 1.27 °C) are similar to previously  
291 reported estrus-related temperature changes. Both Maatje and Rossing (1976) and McArthur et al.  
292 (1992) found that milk temperature increased 0.3 °C around estrus. Other researchers have found  
293 that vaginal temperature increased 0.10 to 1.02 °C around estrus (Lewis and Newman, 1984; Kyle  
294 et al., 1998). Piccione et al. (2003) found that rectal temperatures, though non-automated,  
295 displayed an even greater increases during estrus (1.3 °C). These estrus-related temperature

296 increases have reportedly lasted for  $6.8 \pm 4.6$  h in dairy cows and  $6.5 \pm 2.7$  h in beef cows (Redden  
297 et al., 1993; Kyle et al., 1998).

298 Differences in temperature measurements may have resulted from the difference in  
299 frequency of measurement between the two technologies. The CowManager SensOor sampled  
300 temperature each minute and reported a mean hourly ear surface temperature whereas the DVM  
301 bolus recorded reticulorumen temperature only twice daily at the time the cow entered the parlor  
302 for milking. Reticulorumen temperature readings at those times likely did not accurately  
303 represented the entire 12 hour period between milkings and, therefore, would not be comparable  
304 to ear surface temperature as measured by the CowManager SensOor. Newer versions of the DVM  
305 bolus can continuously monitor temperature, which could reduce variation between the two  
306 technologies.

307 Ear surface temperature as measured by CowManager SensOor was not significantly  
308 influenced by the interactions of DIM at the start of synchronization, parity, or summit milk  
309 production with estrus status. Reticulorumen temperature as measured by DVM bolus was  
310 significantly influenced by the interactions of both DIM at the start of synchronization and parity  
311 with estrus status (Table 2). Cows that started the synchronization protocol at a later DIM  
312 expressed a larger increase in reticulorumen temperature during estrus than cows starting the  
313 synchronization protocol at earlier DIM. Additionally, as parity increased, a smaller estrus-related  
314 increase in reticulorumen temperature was observed. Both of these results contribute to the overall  
315 conclusion that as DIM at the beginning of the synchronization protocol decreased and parity  
316 increased, weaker estrus expression was observed.

317     ***Machine Learning***

318              Because of the low number of observed estrus events in this study ( $n = 18$ ), when 70% of  
319        the data was used for the machine learning training sets, data from only 5 cows was left for the  
320        machine learning testing sets. Consequently, results should be interpreted carefully, keeping in  
321        mind the small sample size. Table 3 shows the sensitivity, specificity, and accuracy accomplished  
322        using different combinations of each of the five technologies and three machine learning  
323        techniques (random forest, linear discriminant analysis, or neural network). Confidence intervals  
324        are reported for each measure of performance to emphasize the difficulty in drawing conclusions  
325        from the small data set.

326              Using the random forest machine learning technique, the CowManager SensOor and  
327        IceQube produced the greatest accuracy (98.6%; Table 3). The CowManager SensOor also  
328        produced the greatest accuracy (100%) when using linear discriminant analysis whereas the  
329        IceQube produced the greatest accuracy (100%) when using neural networks (Table 3). The  
330        number and variety of parameters measured by both the CowManager SensOor (4 parameters  
331        measured) and IceQube (3 parameters measured) likely gave them an advantage in these analysis  
332        over the other technologies which measured only 2 parameters each (HR Tag and Track a Cow).  
333        Similarly, Peralta et al. (2005) showed that although visual observation, activity monitoring, and  
334        mounting detection alone produced low estrus detection sensitivities (49.3%, 37.2% and 48.0%,  
335        respectively), combining all three produced an acceptable sensitivity of 80.2%. Redden et al.  
336        (1993) also found that by combining two parameters (activity and vaginal temperature) that alone  
337        each produced an 80% estrus detection rate, a 90% estrus detection rate was possible.

338              Of the remaining technologies, all machine learning results were similar. Accuracy of the  
339        HR Tag and Track a Cow ranged from 96.6% to 97.9% and from 91.0% to 97.2%, respectively.

340 Compared to other studies that have tested similar machine learning techniques for estrus  
341 detection, these results are high. Krieter (2005) applied the neural network technique, combining  
342 activity and time since last estrus, to a testing set of 74 estrus events. That method accomplished  
343 a sensitivity, specificity, and error rate of 77.5%, 99.6%, and 9.1%, respectively. Mitchell et al.  
344 (1996) applied machine learning techniques to milk yield, milking order, and times since last estrus  
345 data to identify 69% of estrus events in a 44 cow testing set, but experienced a large number of FP  
346 (74%). Both of those analyses predicted the day of estrus, whereas the current study focused on  
347 predicting a 12 h period before estrus. Narrowing the estrus period may be more accurate given  
348 that multiple researchers have found mean estrus duration to be less than 24 h (Kerbrat and  
349 Disenhaus, 2004; Roelofs et al., 2005c; Sveberg et al., 2011). Another explanation for the  
350 improved results in this study is the low number of observations in the testing set. Only 5 cows  
351 were included in the testing set, resulting in a small number of potential TP ( $n = 5$ ), a large number  
352 of potential TN ( $n = 140$ ), and wide confidence intervals.

353 Estrus detection ability of machine learning techniques was superior to visual observation.  
354 When visual observation was compared to P4 results of all 32 cows, a 62.1% sensitivity, 100%  
355 specificity, and 65.6% accuracy of estrus detection were achieved. Non-continuous monitoring  
356 likely limited the ability of visual observation to detect short periods of estrus. Additionally, using  
357 secondary signs of estrus to define estrus rather than standing events alone likely would have  
358 increased estrus detection rate (Roelofs et al., 2005c). The ability to continuously monitor cows  
359 using automated monitoring technologies, allowing detection of short estrus periods and estrus  
360 periods not including mounting, likely contributed to improved performance over visual  
361 observation. However, results should be interpreted carefully given that only 18 cows, all of which  
362 exhibited standing estrus, were included in the machine learning analysis whereas 32 cows, some

363 exhibiting standing estrus and some not, were included in the visual observation results. Cows not  
364 displaying standing estrus could not be included in the machine learning analysis because the study  
365 design did not allow for identification of exact ovulation time.

366 **CONCLUSIONS**

367 Neck activity, ear activity, leg activity, step count, lying bouts, lying time, rumination,  
368 feeding time, and reticulorumen temperature may be useful as predictors of estrus. Ear surface  
369 temperature, as monitored in this study, holds less potential for detecting differences between  
370 periods of estrus and non-estrus. Additionally, applying machine learning techniques to  
371 automatically collected technology data shows potential for estrus detection.

372

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379 graduate and undergraduate students.

380

381   **Table 1.** Comparison of automated monitoring technology<sup>1</sup> parameters (adjusted means  $\pm$  SE)  
 382 during estrus (6 h before and after first observed standing event<sup>2</sup>) and non-estrus (the 14 d before  
 383 estrus).

Category	Parameter	n <sup>3</sup>	Estrus	Non-estrus	P-value <sup>4</sup>
Activity					
	HR Tag neck activity (units/2 h)	18	61.62 $\pm$ 2.04	28.20 $\pm$ 0.78	< 0.01
	IceQube number of steps (per h)	17	300.82 $\pm$ 10.92	79.07 $\pm$ 4.13	< 0.01
	CowManager SensOor high ear activity (min/h)	18	17.40 $\pm$ 0.66	4.25 $\pm$ 0.39	< 0.01
	Track a Cow leg activity (units/h)	18	321.14 $\pm$ 11.87	95.17 $\pm$ 7.16	< 0.01
Lying time and lying bouts					
	IceQube lying bouts (per h)	17	0.35 $\pm$ 0.09	0.72 $\pm$ 0.07	< 0.01
	IceQube lying time (min/h)	17	10.19 $\pm$ 1.91	24.82 $\pm$ 0.95	< 0.01
	Track a Cow lying time (min/h)	14	6.56 $\pm$ 2.55	18.18 $\pm$ 1.81	< 0.01
Rumination and Feeding Time					
	HR Tag rumination (min/2 h)	18	20.47 $\pm$ 2.68	32.96 $\pm$ 0.54	< 0.01
	CowManager SensOor rumination (min/h)	18	12.90 $\pm$ 1.07	22.96 $\pm$ 0.57	< 0.01
	CowManager SensOor feeding time (min/h)	18	16.93 $\pm$ 0.99	8.93 $\pm$ 0.65	< 0.01
Temperature					
	DVM bolus reticulorumen temperature (°C)	18	39.29 $\pm$ 0.21	38.86 $\pm$ 0.18	< 0.01
	CowManager SensOor ear surface temperature (°C)	18	24.17 $\pm$ 1.20	22.97 $\pm$ 0.83	0.20

384   <sup>1</sup>CowManager SensOor, Agis Automatisering, Harmelen, Netherlands; DVM bolus, DVM  
 385 Systems, LLC, Greeley, CO; HR Tag, SCR Engineers Ltd., Netanya, Israel; IceQube, IceRobotics  
 386 Ltd., Edinburgh, Scotland; and Track a Cow, Animart Inc., Beaver Dam, WI

387   <sup>2</sup>Observations for standing estrus occurred for 30 min periods at 0330, 1000, 1430, and 2200 daily

388   <sup>3</sup>Number of cows included in statistical analysis

389   <sup>4</sup>The reported P-value represents the main effect of estrus status (estrus or non-estrus) alone,  
 390 independent of covariate effects

391 **Table 2.** Effect of estrus status<sup>1</sup> (ESTRUS), parity, days in milk at the start of synchronization (DIM), summit milk production  
 392 (SUMMIT), and selected interactions on automated monitoring technology<sup>2</sup> parameters.

Category	Parameter	P-value						
		ESTRUS	PARITY	DIM	SUMMIT	ESTRUS × PARITY	ESTRUS × DIM	ESTRUS × SUMMIT
Activity	HR Tag neck activity (units/2 h)	0.42	0.80	0.01	0.44		< 0.01	
	IceQube number of steps (per h)	0.12	0.03	< 0.01	0.04	< 0.01	< 0.01	
	CowManager SensOor high ear activity (min/h)	0.32	0.82	0.01	0.50		< 0.01	
	Track a Cow leg activity (units/h)	< 0.01	0.01	< 0.01	0.01	< 0.01	< 0.01	< 0.01
Lying time and lying bouts	IceQube lying bouts (per h)	0.64	0.08	0.99	0.25		0.04	
	IceQube lying time (min/h)	< 0.01	0.09	0.73	0.04			
	Track a Cow lying time (min/h)	0.29	0.02	0.24	0.03		< 0.01	
Rumination and feeding time	HR Tag rumination (min/2 h)	0.45	0.02	0.11	< 0.01		0.04	
	CowManager SensOor rumination (min/h)	0.47	0.83	0.09	0.33		< 0.01	
	CowManager SensOor feeding time (min/h)	< 0.01	0.24	0.44	0.84			
Temperature	DVM bolus reticulorumen temperature (°C)	0.38	0.85	0.03	0.48	0.03		0.02
	CowManager SensOor ear surface temperature (°C)	0.20	0.12	0.16	0.13			

393 <sup>1</sup>Observations for standing estrus occurred for 30 min periods at 0330, 1000, 1430, and 2200 daily. Estrus was defined as the 6 h before  
 394 and after the first observed standing event and non-estrus was defined as the the 14 d before estrus.

395 <sup>2</sup>CowManager SensOor, Agis Automatisering, Harmelen, Netherlands; DVM bolus, DVM Systems, LLC, Greeley, CO; HR Tag, SCR  
 396 Engineers Ltd., Netanya, Israel; IceQube, IceRobotics Ltd., Edinburgh, Scotland; and Track a Cow, Animart Inc., Beaver Dam, WI

397 **Table 3.** Estrus detection capability<sup>1</sup> and 95% confidence interval of different automated  
 398 monitoring technologies<sup>2</sup> and machine learning techniques (random forest, linear discriminant  
 399 analysis, and neural network). Machine learning models attempted to identify the 12 h period  
 400 before the first observed standing estrus event<sup>3</sup> from the 28, 12 h periods leading up to observed  
 401 standing estrus. The analysis included 18 cows observed in standing estrus<sup>4</sup>, with 70% used for  
 402 training and 30% used for testing.

Technique	Technology	Sensitivity	Specificity	Accuracy
Random forest	CowManager SensOor	100.00 (47.82 – 100.00)	98.57 (84.93 – 99.83)	98.62 (95.11 – 99.83)
	HR Tag	60.00 (14.66 – 94.73)	99.29 (96.08 – 99.98)	97.93 (94.07 – 99.57)
	IceQube	80.00 (28.36 – 99.49)	99.29 (96.08 – 99.98)	98.62 (95.11 – 99.83)
	Track a Cow	100.00 (47.82 – 100.00)	97.14 (92.85 – 99.22)	97.24 (93.09 – 99.24)
	CowManager SensOor	100.00 (47.82 – 100.00)	100.00 (97.40 – 100.00)	100.00 (47.82 – 100.00)
	HR Tag	100.00 (47.82 - 100.00)	97.86 (93.87 – 99.56)	97.93 (94.07 – 99.57)
	IceQube	100.00 (47.82 – 100.00)	97.86 (93.87 – 99.56)	97.93 (94.07 – 99.57)
	Track a Cow	100.00 (47.82 – 100.00)	96.43 (91.86 – 98.83)	96.55 (92.14 – 98.87)
	CowManager SensOor	100.00 (47.82 – 100.00)	98.57 (94.93 – 99.83)	98.62 (95.11 – 99.83)
	HR Tag	100.00 (47.82 – 100.00)	96.43 (91.86 – 98.83)	96.55 (92.14 – 98.87)
	IceQube	100.00 (47.82 – 100.00)	100.00 (97.40 – 100.00)	100.00 (97.49 – 100.00)
	Track a Cow	100.00 (47.82 – 100.00)	90.71 (84.64 – 94.96)	91.03 (85.16 – 95.14)

403 <sup>1</sup>Sensitivity = TP/(TP + FN), specificity = TN/(TN + FP), accuracy = (TP + TN)/(TP + TN + FP  
 404 + FN); TP = true positive, TN = true negative, FP = false positive, and FN = false negative

405 <sup>2</sup>CowManager SensOor, Agis Automatisering, Harmelen, Netherlands; HR Tag, SCR Engineers  
 406 Ltd., Netanya, Israel; IceQube, IceRobotics Ltd., Edinburgh, Scotland; and Track a Cow, Animart  
 407 Inc., Beaver Dam, WI

408 <sup>3</sup>Observations for standing estrus occurred for 30 min periods at 0330, 1000, 1430, and 2200 daily

409 <sup>4</sup>Data from only 14 cows was used for Track a Cow lying time and data from only 17 cows was  
 410 used for all IceQube parameters

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518 **Figure 1.** Timeline of synchronization injections, blood sampling (BS), and visual observation (VO) for cows used in a study testing 5  
519 automated monitoring technologies' estrus detection capabilities. The synchronization protocol was a modified G7G Ovsynch with  
520 injections given at 0800. Two injections of PGF<sub>2α</sub> (6 h apart; 0800 and 1400) were administered on d 0. Blood sampling was conducted  
521 at 0800. Visual observation was conducted 4X for 30 min periods at 0330, 1000, 1430, and 2200.  
522

