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Vaginal temperature and collar activity variations in relation to fecal progesteragens of captive moose

Jennifer Høy-Petersen^{1,2*}, Lucie Lemière¹, Dan P. Thompson³, Alexandra Thiel¹, John A. Crouse³, Erik Ropstad², Anne Randi Græsli¹, Jon M. Arnemo^{1,4} and Alina L. Evans¹

Abstract

Background Timing of reproductive events can be crucial for a species' population growth and stability. Accurate detection of reproductive phenology presents a challenge to scientists studying wild species, including moose (*Alces alces*). Currently, there are several established methods for monitoring reproductive activity and events in domestic ruminants, including the use of biologging devices. The main objective of this study was to determine whether female moose display a distinct thermal and activity pattern associated with luteal activity during the estrous cycle, which could be used to determine the onset of their breeding season. We deployed biologging devices and collected fecal samples from 12 captive female moose on the Kenai Peninsula, Alaska, USA to explore variation in vaginal temperature and collar activity and the relationship between these variables and fecal progesteragen concentrations. Fecal samples were collected from mid-August to mid-October and analyzed using radioimmunoassay to determine the concentration of fecal progesteragens to classify luteal activity.

Results Captive female moose displayed an identifiable thermal pattern during the onset of luteal activity from mid-September to mid-October, associated with the initial estrous cycle of their breeding season. In contrast, we did not observe a distinct pattern in activity during this period. Recurring patterns in both vaginal temperature and activity were identified between mid-October and mid-November, however, which were likely associated with subsequent estrous cycles but not included in our fecal sampling period.

Conclusions This study supports that female moose display an identifiable pattern in vaginal temperature which is associated with luteal activity of the initial estrous cycle of the breeding season. An identifiable pattern was observed for both vaginal temperature and activity registrations at the presumed timing of subsequent estrous cycle of the breeding season.

Keywords Moose, *Alces alces*, Alaska, Body temperature, Activity, Biologger, Breeding season, Estrous cycle, Reproduction, Fecal progesteragen

*Correspondence:

Jennifer Høy-Petersen

jhoypetersen@gmail.com

Full list of author information is available at the end of the article



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Background

Understanding how environmental factors affect populations of wild species and predicting the expected ecological impacts is crucial to consider in management and conservation of wildlife [1]. Environmental fluctuations caused by climate change may shift the timing of phenological events and consequently impact the general reproductive success and rate of survival of wild animals and plants [2]. Different moose (*Alces alces*) populations are experiencing fluctuations, and recent studies show how populations of moose living in the southern ranges of their habitat are declining, with a reduced calf survival rate [3, 4], while other populations appear to be relatively stable [5], expanding to new areas [6], or increasing in size [7, 8]. Studies in Scandinavian [9–11] and North American [12–14] moose have shown that seasonal climatic changes, physical condition, fertility problems, predation, forage nutritional quality and availability impact reproductive success and calf survival. It has been hypothesized that calves which are conceived later in the breeding season (i.e., not during the first estrous cycle) may be born when forage quality and availability are sub-optimal, resulting in reduced calf growth and probability of survival [15]. Furthermore, calves born later are likely more vulnerable to predation [16]. Thus, a deeper understanding of the reproductive phenology and physiology of moose would benefit research aimed at studying population fluctuations.

The estrous cycle and breeding season in Scandinavian and North American moose is reported to occur from early September and into November [17–19]. Moose are seasonal breeders [3], meaning that they only breed during a specific time of the year. Changes in day length (photoperiod) affect endocrine signaling pathways which are known to regulate the transition from reproductive inactivity (anestrus) to reproductive activity (estrus) in seasonal breeders [20]. Sexual maturation in female moose is speculated to occur after 1.5 years of age but varies between different cohorts and their respective environmental conditions [3, 17, 21–23]. Peak ovulation dates for Scandinavian moose [21] are estimated to be synchronized and occur in late September and early October with regional variations, which align well with when the majority of mating events occur during the rut in North American moose [24]. Schwartz et al. [17, 25] estimated each estrous cycle to last about 24 (range = 22–28) days and a possible recurrence of 4–7 cycles during the breeding season. Duration of sexual receptivity, or estrus, is limited to 1–36 h at the beginning of each cycle. Malmsten et al. [18] found that for a limited number of female moose mating had likely occurred 2 weeks into pregnancy, meaning that copulation may not be a reliable indicator of estrus. In addition,

the timing and relation between observed estrus and ovulation are not yet known for moose, so it is not possible to estimate a day of ovulation solely based on external signs of estrus. It is, however, likely that ovulation occurs during or shortly after estrus [3].

Today, we have well-established methods for detecting and monitoring reproduction in domestic ruminants, including behavior or physiological observations, measuring progesterone hormone, or monitoring variables, such as body temperature and activity [26, 27]. In contrast, we have less experience and few established methods for monitoring the estrous cycle in wild animals, including moose. As behavioral cues in wild ungulates may be less prominent, the reliability of behavioral observations to determine or validate reproductive events in wild species are considered to be less accurate [28]. This further supports the need for more accurate reproduction monitoring techniques in wildlife. Captive wildlife is more habituated to human presence, which facilitates the feasibility and accuracy of reproduction monitoring and decreases capture-related stress. Therefore, reproduction studies in wild species have initially been conducted in captive animals, before being extended to free-ranging ones [29].

To overcome practical and ethical challenges associated with studying non-domesticated animals, less-invasive methods are essential. Measuring fecal steroid hormones associated with reproductive physiology offers an alternative to the more invasive method of acquiring a blood sample to monitor reproduction in wildlife species. Progesterone is a steroid hormone associated with the estrous cycle and is produced by the corpus luteum in the ovary before it is released into the blood, where it circulates either freely or bound to plasma proteins. Further metabolism occurs in the liver, before excretion as conjugates in urine, bile (and subsequently feces), and saliva [30]. Measuring progesterone metabolites in feces (i.e., fecal progestagens) is a widely used method to monitor reproductive physiology in several wild ruminant species [31–35], including moose [25]. As an illustration, estrus in captive moose has been suggested to occur on average 0.6 days prior to the nadir (95% confidence interval (95% CI) = 4 days before to 3 days after the nadir) in fecal progestagen levels, by visual confirmation of estrus such as copulation with a bull or ruffled rump hairs as an indicator of having been mounted [17, 25].

Previous monitoring studies have shown that moose display seasonal patterns of body temperature and activity [36, 37]. In general, body temperature decreases from July to September in Scandinavian moose [36], and from July to October in Alaskan moose [37], and increases from April to June [36, 37]. In Scandinavian moose, activity is highest in June and lowest in February [36]. These

patterns are likely associated with seasonal hypometabolism. Moreover, biologging devices have recently been used to investigate patterns in body temperature and activity during pregnancy and parturition in wild Swedish moose [38], revealing that body temperature is higher in pregnant moose compared to non-pregnant moose [37]. Furthermore, monitoring the combination of body temperature and activity changes can help detect the time of calving, an event associated with a drop in both variables [39]. These findings illustrate that moose show identifiable patterns in body temperature and activity related to reproductive events, similar to what has been reported in domestic cattle [40–42]. However, a precise method to accurately determine the onset of estrous cyclicity in free-ranging moose is yet to be established and would increase researchers' abilities to evaluate consequences of a shift in reproductive phenology, such as estrous cyclicity, breeding, and conception.

This study describes the application of novel reproductive monitoring techniques in female moose, with a general aim to broaden our knowledge about their reproductive phenology and physiology.

The main aim of this study was to explore whether vaginal temperature and collar activity data from captive female moose could be used to determine the onset of their breeding season. In dairy cattle it has been documented that body temperature decreases just prior to estrus, and then sharply increases on the day of estrus [40, 43]. Following this peak there is a decrease around the expected time of ovulation before a steady increase post-ovulation, during the luteal phase. The rise in body temperature around estrus has been attributed to the thermogenic effect of progesterone [42], increased vaginal blood flow [44], and the accompanying increase in activity [40]. An increase in activity is associated with the timing of estrus in dairy cattle [45–48], and in wild ungulate species, such as white-tailed deer (*Odocoileus virginianus*), where peak daytime movement usually occurs on the day of mating or the day before [49]. Based on these findings and use of similar methodology, we made the following predictions related to the estrous cycle in moose. Since it has been found that fecal progestagen values can be utilized to classify luteal activity during the estrous cycle in moose [25], and the study area and animals enabled daily fecal collection, we predicted that this variable could be used to detect and confirm the initial luteal activity of the breeding season (P1). In addition, we predicted that there would be an identifiable pattern in vaginal temperature (P2) and collar activity (P3) associated with luteal activity in moose. Our expectations for P1 through P3 were that the three variables would share similarities with physiological changes which are associated with specific parts of the estrous cycle and ovarian

activity in dairy cattle; a low basal level of plasma progesterone preceding the onset of luteal activity, combined with an increasing trend in vaginal temperature and a peak in activity [50]. Dairy cattle were selected as a reference species for the physiological changes that occur during the estrous cycle due to the amount of rigorous research on validation of activity and body temperature to detect ovarian activity which is available compared to other ungulates that may share more similarities with moose.

Methods

Study area and animals

We collected data registrations from 12 captive female moose, ages 2–19 years, at the Kenai Moose Research Center, on the Kenai Peninsula, Alaska, USA. The Kenai Moose Research Center is operated by the Alaska Department of Fish and Game and is located on the Kenai National Wildlife Refuge (60°43' N, 150° 26' W). The study area is characterized as boreal forest, including dense forests, wetlands, and open meadows. Present tree species are dominated by white spruce (*Picea glauca*), black spruce (*Picea mariana*), Alaska birch (*Betula neoalaskana*), Scouler's willow (*Salix scouleriana*), and quaking aspen (*Populus tremuloides*). The study animals were kept outdoors in two 2.6 km² enclosures with free access to water and natural forage, which enabled the desired frequency of individual-specific fecal sample collection. Each individual had a specific color combination of duct tape on their GPS collar for identification and fecal pellets were only collected after confidently identifying the animal and defecation had been observed. The fence kept the study animals physically separated from wildlife in the surrounding area. Occasionally wild bulls were observed along the fence lines of both enclosures and one of the enclosures also shared a small section of fence with a pen that housed three adult bulls, both of which allowed a certain degree of indirect contact between females and males.

In May and July 2021, the captive female moose were immobilized following an earlier described protocol [37] with a combination of Thiafentanil oxalate (0.001–0.004 mg/kg estimated body mass; 10 mg/mL; Wildlife Pharmaceuticals Inc., Windsor, CO, USA) and Xylazine (0.03–0.05 mg/kg estimated body mass; 100 mg/mL; Lloyd Laboratories, Shenandoah, IA, USA) by intramuscular hand-injection and equipped with Vertex Plus Global Positioning System (GPS) collars (Vectronic Aerospace GmbH, Berlin, Germany) and Vaginal Implant Transmitters (VITs; size=big; Vectronic Aerospace GmbH; Berlin, Germany). Immobilization was reversed by a combination of Atipamezole HCl (0.005 mg/kg estimated body mass; ¼ dose intravenous, ¾ dose

intramuscular; 5 g/mL; Zoetis, Parsippany, NJ, USA) and intramuscularly administered Naltrexone HCl (100 mg/mg Thiafentanil oxalate intramuscular; 50 mg/mL; Zoo-Pharm LLC, Laramie, WY, USA). GPS collars and VITs were manually removed from all animals without sedation on November 23rd, 2021.

Vaginal temperature and collar activity

The VITs transmitted vaginal temperature (T_v ; °C) every 5 min to the GPS collar and were additionally programmed to record vaginal temperature and store this data on-board every 17:10 min as a backup should transmission fail. During the aforementioned immobilization procedure, a VIT was inserted into the vaginal canal of each individual using a lubricated (OB Lube; Jorgensen Laboratories Inc., Loveland, CO, USA), sterilized speculum (Sterile Disposable Vaginal Speculum; Jorgensen Laboratories Inc.) following previously established procedures [51]. T_v was successfully downloaded from 11 individuals (from GPS-collar $n=10$ and from the VIT $n=1$). One VIT stopped recording on the 12th of August 2021 (i.e., before the start of fecal sample collection), and so this individual was excluded from further T_v analysis. Over 150,000 registrations of T_v were made during the dates with corresponding fecal progesterone values (i.e., August 23rd to October 15th, 2021). Daily mean T_v was calculated, resulting in 594 registrations during this period.

The GPS collars included a triaxial accelerometer which registered forward–backward, left–right, and up–down movements, stored as X, Y, and Z, respectively. Movement was recorded in 5-min intervals as average values of each axis ranging between 0 and 255 at 6–8 Hz and stored in the collar. Overall activity was calculated by summing the X, Y and Z axes, giving values ranging from 0 to 765 every 5 min, with 0 representing no or low activity and 765 the highest activity level. Activity registrations were successfully stored for all 12 individuals resulting in over 185,000 registrations for each axis (X, Y, and Z) during the period of fecal sampling (i.e., August 23rd to October 15th, 2021). Daily summed activity during the fecal sampling period was calculated, resulting in 234 to 288 registrations a day.

VIT validation

We validated the accuracy and precision of the VIT temperature logger within the range of moose body temperature (i.e., within the range of vaginal temperature in this study and prior research [37]). We placed the VITs in a warm water bath heated to 37.00 °C, and we then increased the temperature of the water bath by 0.50 °C until 40.50 °C. The water bath temperature was measured with a National Institute of Standards and

Technology-certified thermometer (Traceable® Digital Thermometer Model 90080–09, accuracy ± 0.05 °C, 0 to 100 °C, resolution 0.001 °C (Webster, TX, USA)). We also tested the accuracy and precision of the VIT in an ice bath, which has been traditionally used to calibrate temperature logger VITs [52]. We used linear mixed model regression, with individual VIT as a random intercept, to determine accuracy of the loggers.

Fecal samples

A total of 468 fecal samples were collected from mid-August to mid-October 2021. The study period was selected based on previous literature on the timing of the species' reproductive season, personal observations of the study population's estrous behavior in previous years, and a desire to collect samples during the transition between anestrus to estrus [17, 21, 24]. Individual samples were collected every other day from the 23rd of August to the 21st of September 2021. After this, samples were collected daily until the 16th of October 2021. All sampling occurred between 07:00 and 19:00 Alaska Daylight Time. Each sample weighed between 100 and 200 g and was collected in pre-labeled Whirl–Pak bags (Nasco Whirl–Pak®, Fort Atkinson, WI, USA) during or shortly after defecation and stored on ice in a cooler bag, before being transferred to a -18 °C propane freezer. The frozen samples were freeze dried (Labconco model 7752020, Kansas City, MO, USA) and homogenized to a powder to further distribute metabolites evenly before randomly subsampling 5 g [53]. The subsamples were shipped to an endocrine laboratory (Applied Biosciences, TX, USA) for further extraction and analysis, following a previously described protocol [54]. Progesterone concentration was determined by radioimmunoassay (RIA; Catalog #07-270102; ImmuChem Double Antibody, 125I RIA Kit, MP Biomedicals, Costa Mesa, CA, USA), following the manufacturer's protocol. Intra-assay variation was 10.6% on a low pool, medium pool at 7.93%, and 5.21% on a high pool. The extraction efficiency average on spiked samples of fecal material was 83.23% ($n=10$). Fecal progesterone concentration is expressed as nanogram (ng) of immunoreactive fecal progesterone hormone metabolites per gram (g) dry fecal weight.

Environmental variables

Within the Kenai Moose Research Center there is a National Oceanic and Atmospheric Administration (NOAA), U.S. Climate Reference Network weather station which recorded environmental variables, including ambient air temperature (T_a , °C), relative humidity (%), solar radiation (W/m^2), windspeed (m/s), and precipitation (mm) every 5 min [55]. Ambient air temperature and relative humidity recorded by the NOAA weather station

were used to calculate dew point temperature ($^{\circ}\text{C}$), which was further used to calculate actual vapor pressure (hPa) [56]. The NOAA weather station had 1 day of missing data for ambient temperature, relative humidity, and solar radiation (25th of August, 2021). HOBO dataloggers (HOBO U23 Pro v2 Temperature/Relative Humidity datalogger; HOBO Pendant Temperature/Light 64 K datalogger; Onset Computer Corp., Pocasset, MA, USA) were deployed adjacent to the NOAA weather station for the duration of the study. We used simple linear regression to estimate the missing NOAA values from the HOBO logger data for daily mean ambient air temperature ($y = 0.96 * T_a + 0.50$, $r^2 = 0.99$), daily range in ambient air temperature ($0.88005 * T_a R + 0.49969$, $r^2 = 0.98$) relative humidity ($y = 1.05859 * RH - 9.79112$, $r^2 = 0.98$), and solar radiation ($y = 0.0004 * \text{lux} - 0.1266$, $r^2 = 0.98$).

Data preparation and analysis

All data handling and statistical analysis was performed in R version 4.1.0 (R Core Team, 2021), using RStudio version 1.4.1717. Means are reported with \pm SD in parenthesis. T_v and collar activity data were filtered for the 3 days following capture to exclude values which would likely be elevated due to lingering effects of the immobilizing drugs or capture-related stress [37]. Since fecal collections occurred on alternate days for the first half of the sampling period, fecal progesterone values were linearly interpolated using the “approx” function from the “zoo” package [57] in R to generate daily values to classify luteal activity. Similarly, daily values for T_v and collar activity were generated by calculating the daily mean T_v and the total daily sum of activity values (referred to as the daily sum of activity).

To determine a baseline level of fecal progesterone considered as indicative non-luteal activity, we calculated the mean of the 2 lowest values for each individual. Furthermore, a threshold was created by multiplying the baseline level by 2, to differentiate between non-luteal activity and luteal activity. If fecal progesterone levels were below this threshold for at least 1 day, and then increased, and stayed above it for at least 14 days, we defined the day on which the threshold was exceeded as an onset of luteal activity (OLA), and the period during which fecal progesterone levels stayed above the threshold as luteal activity. Because the duration of the luteal phase is currently unknown for moose we based our OLA classification method on existing approaches for classifying normal luteal activity in cattle [58], along with the average luteal phase length in other seasonal breeding ruminants, such as marsh deer (*Blastocerus dichotomus*), domestic sheep (*Ovis aries*), and domestic goats (*Capra hircus*; 15, 14 and 16 days, respectively [34, 59, 60]). The date of the first OLA was assigned as day 0, which was used as a scale to

explore variations in T_v and collar activity for that individual in relation to luteal activity. The above calculations and classifications were done by applying a rolling function using the “zoo” R package [57].

The four moose which we were not able to determine an OLA for either had erratic fecal progesterone profiles (age = 2 years), fecal progesterone levels which did not stay above the set threshold for the minimum required days, or an erratic fecal progesterone profile which stayed above the set threshold but was not preceded by a day in which the level was below the set threshold (age = 19 years). T_v and activity registrations from these individuals were, therefore, excluded from further statistical analyses. These individuals were either below or above prime age (2.5–10.5 years [21]), indicating that our method is more successful when applied on prime aged moose.

Relationship between luteal activity and vaginal temperature

To determine when changes in daily mean T_v occur in relation to the onset of luteal activity, and to account for non-linear relationships between response and explanatory variables, we used Generalized Additive Mixed Models (GAMMs; using the “mgcv” R package from Wood [61]). All explanatory variables included in the model were adjusted to daily values to match up with the daily frequency of fecal progesterone values. Daily mean T_v was selected as the response variable and OLA was included as the main explanatory variable to explore the relationship to luteal activity. In addition, explanatory variables that are known to influence core body temperature in moose were included: daily sum of activity, ordinal day (to account for seasonal variation in T_v [36]), daily mean T_a ($^{\circ}\text{C}$), daily range of T_a ($^{\circ}\text{C}$), daily mean vapor pressure (hPa), daily mean wind speed (m/s), total daily precipitation (mm), and total daily solar radiation (W/m^2) [37]. Correlation and structure of the explanatory variables were checked using the function “ggpairs” from the “GGally” [62] extension to the “ggplot2” R package [63]. If the Pearson’s correlation coefficient between OLA and another explanatory variable was above 0.50 or below -0.50, it was considered a high correlation [64] and this variable was not included together with OLA in the same model (this was the case for ordinal day, vapor pressure and daily ambient temperature, see Additional file 1). In addition, if two explanatory variables other than OLA had a correlation with each other above or below this threshold, the combination of these variables was not included in the same model (this was the case for daily ambient temperature and solar radiation, and wind speed and relative humidity, see Additional file 2).

These steps left us with the following explanatory variables: OLA, daily range of T_a , daily mean relative humidity,

total daily precipitation, daily mean wind speed, total daily solar radiation, and daily sum of activity (Additional file 9: Table S1). In addition, to test if the trend in T_v was attributed to seasonal variation rather than OLA, we included ordinal day in one of the models. We included the individual moose (“CollarID”) as a random intercept in all models to control for inter-individual variability and repeated measurements. A gaussian distribution with an identity link function and the maximum likelihood was used. We then ran a model selection based on Akaike’s Information Criterion, adjusted for small sample sizes (AICc, function “AICctab” from the “bbmle” R package [65]), by selecting the most parsimonious model within $\Delta\text{AICc} \leq 2$ [66]. Residuals were assessed and basis dimensions for the parameter k were checked before we inspected diagnostic plots to validate model assumptions. Finally, the selected model was refitted with restricted maximum likelihood.

Relationship between luteal activity and collar activity

GAMMs were also used to determine when changes in daily sum of activity occur in relation to the onset of luteal activity. Daily sum of activity was selected as the response variable and OLA was included as the main explanatory variable to explore the relationship between luteal activity and activity values. The same environmental variables mentioned previously were included as they have been shown to affect behavior and habitat selection in moose [67]. In addition, we included daily mean T_v as an explanatory variable since thermoregulation influences activity levels in moose [67], and the same random intercept (individual moose, “CollarID”). Due to positive-skewed data we used a gamma distribution with a log link function and applied the same model selection process as described above.

Results

Classification of luteal activity

Fecal progesterone metabolite concentrations observed in the 468 fecal samples ranged from 2.20 to 68.30 ng/g fecal matter ($15.30 \text{ ng/g} \pm 9.30 \text{ ng/g}$; Additional file 9: Table S2). The classification method used to differ between luteal activity and non-luteal activity was able to identify a date for the onset of luteal activity (OLA) for 8 out of 12 individuals (Fig. 1). The OLA had a mean date of occurrence on the 23rd of September (± 4 days) and showed no significant interindividual variability (ANOVA, $P=0.59$).

Vaginal temperature and collar activity

Vaginal implant transmitters (VIT) placed in a warm water bath ($37.00\text{--}40.50 \text{ }^\circ\text{C}$) had an accuracy of $0.25 \text{ }^\circ\text{C}$ (95% CI $0.23\text{--}0.27 \text{ }^\circ\text{C}$), and the variance associated

with the random intercept of VIT was insignificant ($\sigma^2 < 0.01$). When the VITs were placed in an ice bath, we found that the accuracy was much lower at $1.23 \text{ }^\circ\text{C}$ (95%CI $0.33\text{--}2.15 \text{ }^\circ\text{C}$), and the variance of the random intercept of VIT was significant ($\sigma^2=1.26$). In both water baths, the VITs registered temperatures that were colder than the actual temperature of the water bath. Across all individuals, the registered daily mean vaginal temperature (T_v) declined from late August to late September. From late September to early October periodic variations in daily mean T_v were observed (i.e., a discernible increase over a two to 3-week period followed by a 1-day decrease). Each moose had at least one such characteristic T_v pattern during the fecal sampling period. Additional patterns in T_v were observed for eight individuals on dates exceeding the fecal sampling period. The interval between the first and second observed pattern in T_v was significantly shorter than the interval between the second and third observed pattern (paired T test, $P=0.02$, 24 ± 2 days vs. 27 ± 2 days, respectively).

In general, collar activity (referred to as the daily sum of activity, see Methods) declined from August towards November. Two moose had discernible but relatively small peaks of activity during the fecal sampling period. However, a total of nine moose had one or two more obvious activity peaks from mid-October until the end of activity registrations in November, which were found 27 ± 1 day apart from each other.

For the seven moose which had T_v , collar activity, and an OLA available during the fecal sampling period, the general observation was that T_v started to increase a few days before or after day 0 of OLA (range = 7 days before to 3 days after OLA), whereas activity values did not appear to show as much of a distinct pattern around this time (Fig. 2, for the remaining individuals see Additional file 3, Additional file 4, Additional file 5, Additional file 6, Additional file 7, and Additional file 8). Activity peaks were 1.40 to 2.70 times greater (1.80 ± 0.40) than the individual mean of activity values during the fecal sampling period.

On dates exceeding the period of fecal sample collection (i.e., after October 15th), we observed that the drop in T_v would periodically align with a peak in activity (a total of 15 times, two times for six individuals and one time for three individuals). The peak in activity was generally seen on the same day as the drop in T_v , or 1–2 days after, with an interval of 27 ± 2 days. Activity peaks were 2.30–6.80 times greater (4.00 ± 1.40) than the individual mean of activity values for this period, which was significantly higher than for the previous period that included corresponding fecal progesterone values (paired T test, $P < 0.01$).

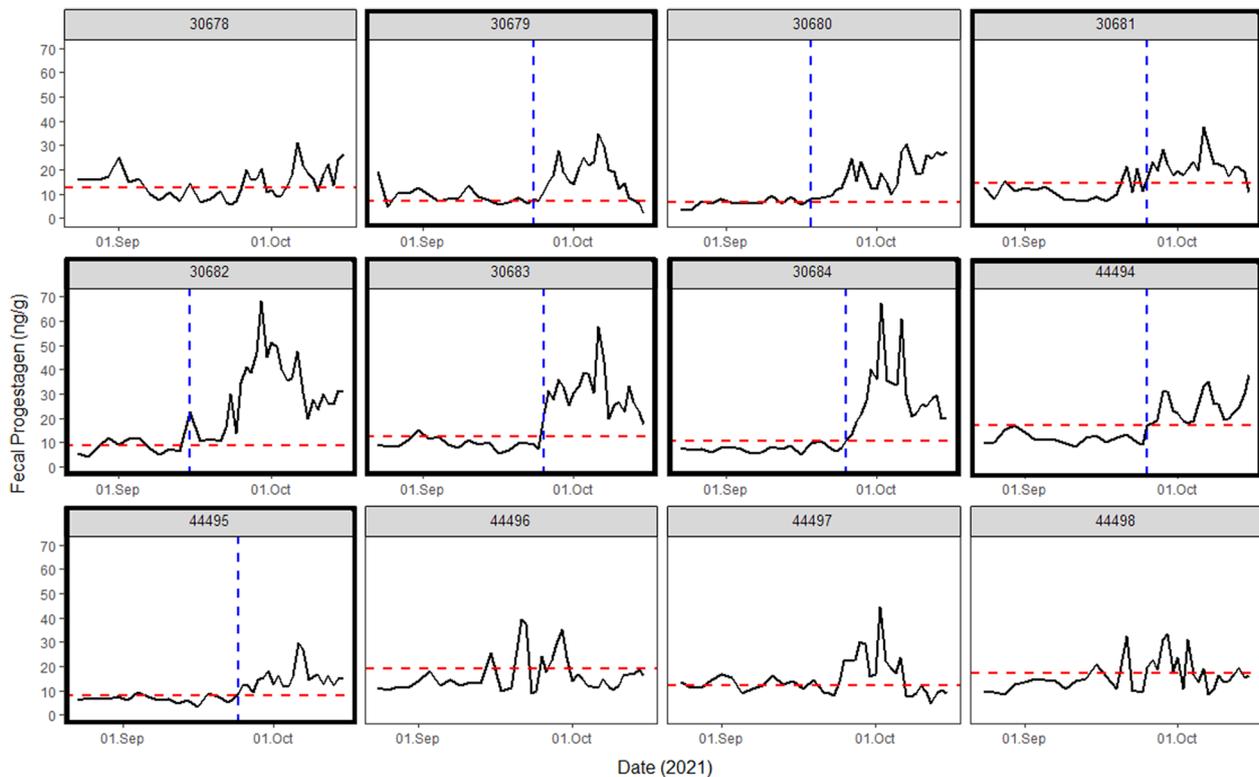


Fig. 1 Fecal progesterone profiles. Fecal progesterone (ng/g) profiles from captive female moose ($n = 12$) from August 23rd to October 15th, 2021, at the Kenai Moose Research Center, AK, USA. The bold outlined graphs indicate the seven individuals with fecal progesterone profiles which fulfill the criteria to determine a date for the onset of luteal activity (OLA). The remaining five graphs are for moose with fecal progesterone profiles which did not fulfill the criteria to determine a date for OLA or did not have vaginal temperature data for the whole duration of the study period (moose 30680) and was, therefore, excluded from further data analysis. Dashed red horizontal line = individual progesterone threshold. Dashed blue vertical line = date for OLA

Relationship between luteal activity and physiological changes

Registrations from a total of seven moose which had T_v , activity and an OLA available were included in the statistical models. The highest ranked model for predicting T_v included only the explanatory variable OLA which explained 72.30% of the deviance (Additional file 9: Tables S3 and S4). This model predicted that T_v dropped by 0.18 °C over a 17-day period, before stabilizing at 37.77 °C (95% CI 37.74–37.80 °C) 5 days before OLA (Fig. 3). Subsequently, T_v increased by 0.17 °C over a second 17-day period before entering a new decreasing phase after reaching 37.94 °C (95% CI 37.91–37.97 °C) 12 days after OLA.

The highest ranked model for predicting activity included the explanatory variables OLA and total daily precipitation (Additional file 9: Tables S5 and S6) which explained 39.30% of the deviance. This model predicted that activity decreased by 19.87% from 15 days before OLA (95% CI 6666.09–7910.89) to 12 days after OLA (95% CI 5340.82–6339.85), and then increased by 13.44%

from 12 days after OLA to 20 days after OLA (95% CI = 5959.89–7582.65). In addition, when daily mean precipitation increased from 0.00 mm to 17.80 mm, activity increased by 15.04% (0.00 mm 95% CI 5902.4–6979.4, 17.80 mm 95% CI 6579.27–8673.83).

Discussion

The goal of this study was to gain better insights about the moose estrous cycle and investigate the timing of luteal activity using biologging technology. We observed periodic patterns in T_v from mid-September and into November in prime-aged moose (2.5–10.5 years [21]). Patterns in collar activity were visible in October and November (supporting P3) but were less obvious or absent in September (not supporting P3), during the time when fecal progesterone data were collected. There continue to be several aspects regarding the estrous cycle of moose which are yet to be described but our results align well with existing literature on the reproductive characteristics of moose [3, 17, 18, 21] and provide new

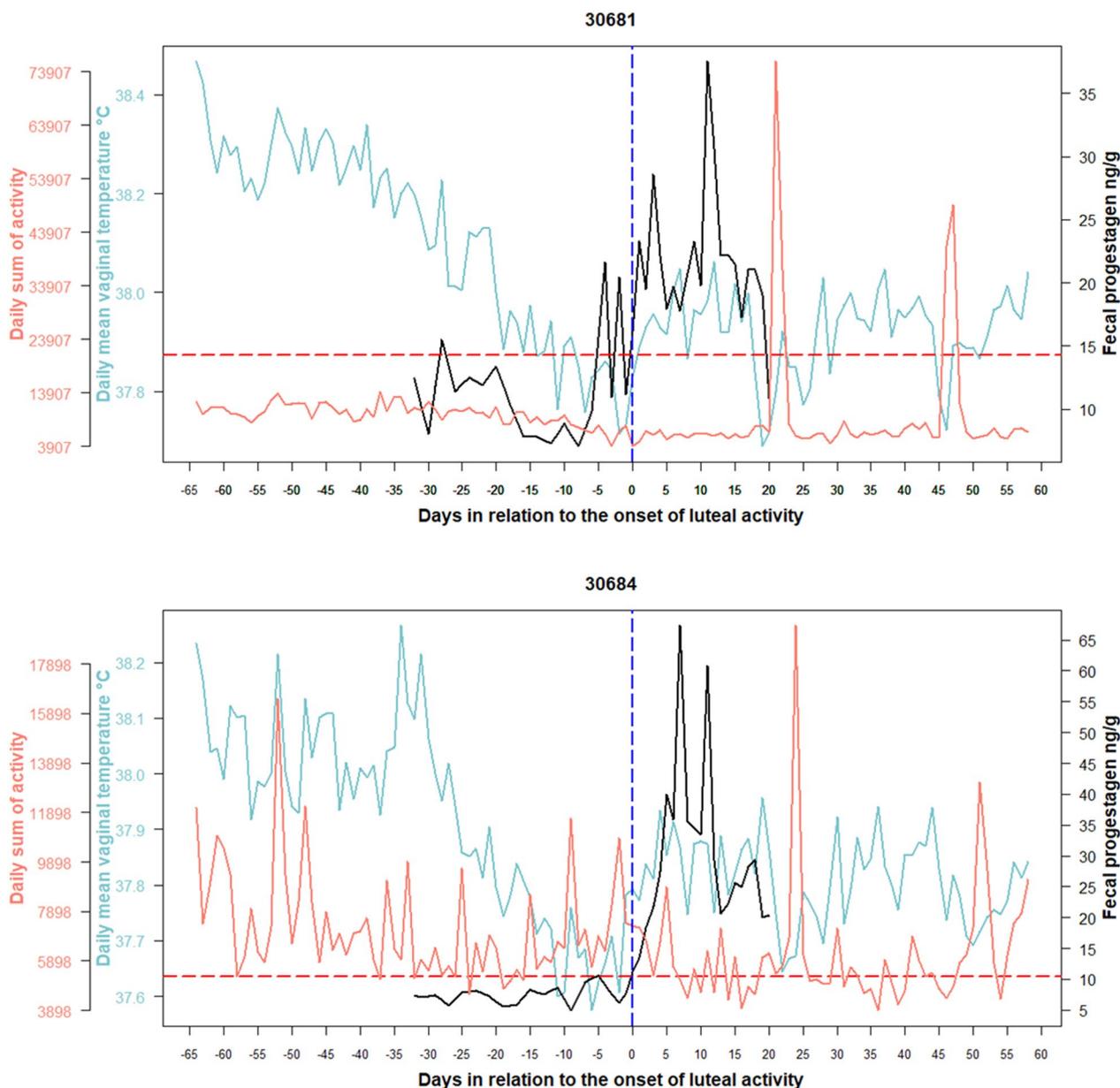


Fig. 2 Vaginal temperature, collar activity, and fecal progesterone. Graphs from two captive female moose that were 9 years (IDs = 30681 and 30684) containing daily values for mean vaginal temperature (blue line) and daily sum of activity (red line) recorded between July 23rd and November 22nd, 2021, and fecal progesterone concentrations recorded between August 23rd to October 15th, 2021. Dashed vertical blue line = onset of luteal activity, which is on the 23rd of September for both graphs. Dashed horizontal red line = individual fecal progesterone threshold

perspectives on physiological variables during the breeding season and their relation to the estrous cycle.

As anticipated, we were able to classify the initial luteal activity of the estrous cycle in captive moose based on fecal progesterone values (P1). Since the study period lasted for 54 days, combined with cycle length in moose (22–28 days [17]), we predicted that we would observe at least one estrous cycle per moose, as well as

the transition from seasonal anestrus to estrus. Based on the early timing of fecal sample collection (i.e., starting date on the 23rd of August), we likely captured the first estrous cycles of the season, as cycling in moose has been reported to occur between September and November [17–19, 21, 24]. This assumption is strengthened by the concentrations of fecal progesterone during the first half of the sampling period (i.e., from the 23rd of August until

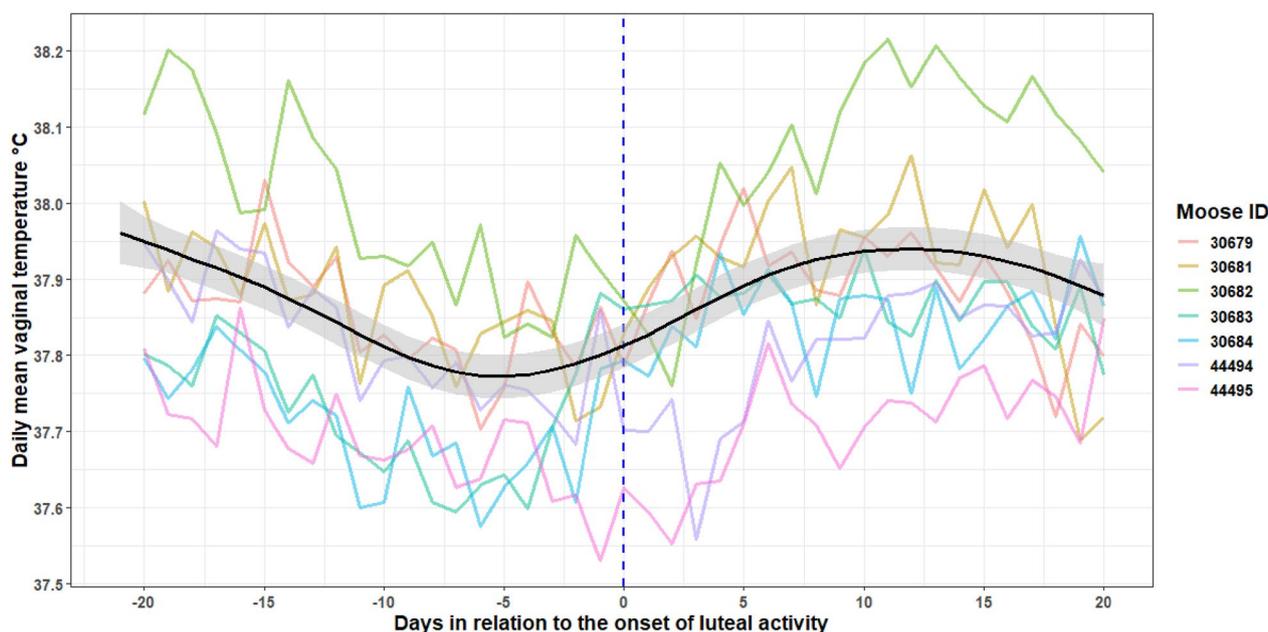


Fig. 3 Model predictions. Predicted values of a generalized additive mixed model predicting daily mean vaginal temperature from captive female moose plotted against days in relation to the onset of luteal activity (OLA). Black line = the predicted daily mean vaginal temperature over time. Shaded gray ribbon = 95% confidence interval. The colored lines represent the raw data that the model based its predictions on, and each color indicates each moose that contributed to the data (n = 7). Dashed vertical blue line = day 0 for OLA

mid-September). During this time, progestagen levels stayed below or near the individually calculated threshold and thus deviated from the expected periodic pattern that is associated with multiple estrous cycles in captive moose [25].

We found that the timing of OLA was not significantly different between individuals, which supports the theory that female moose are synchronized in respect to estrous cyclicity [21, 25]. In Scandinavia, 95% of ovulations in wild moose have been reported to occur within an interval of less than 10 days from late September to early October [21]. On a global scale, most mating events in moose have been reported to occur over a 15-day period, from the 23rd of September to the 8th of October [24]. The mean date for OLA in the present study (the 23rd of September) also corresponds well with previously reported dates for the estrous cycle and breeding season in moose [17–19, 21, 24].

Monitoring progesterone concentrations alone is not considered sufficient to predict the specific timing of ovulation in cattle [68]. Therefore, without a method to specifically determine if the study animals were in estrus, such as observing behavioral cues like copulation with a bull [25], measuring luteinizing hormone (LH), or examining the ovaries through transrectal palpation and/or ultrasonography [69, 70], we have taken care not to draw any assumptions regarding estrus or ovulation. Our focus

was to use the data to identify when progestagen concentrations were indicative of luteal activity. Future studies should include additional methods to estimate the timing of estrus and ovulation in captive moose, such as registration of behavioral cues, directly through observation or indirectly using devices that detect when a female is mounted [71]. Another strategy could be the application of remote blood collection technology [72–75] in captive moose to directly measure levels of hormones associated with the estrous cycle (i.e., LH, progesterone, estradiol, and follicle-stimulating hormone [76]).

The VITs used in this study had a lower accuracy than what was reported by the manufacturer (0.25 °C versus 0.10 °C, respectively). Furthermore, although calibration in ice water has previously shown no need for adjustment for this VIT model [52], we found this was not a good method for evaluating the accuracy of the VITs and so we recommend that temperature loggers are validated within the range of body temperatures being measured in the species of study. The observed inaccuracy should be taken into account when interpreting our results. When exploring patterns in T_v and daily sum of activity in relation to fecal progestagen and luteal activity, our expectations were met to a certain degree (P2 and P3). An increase in daily mean T_v was observed around the same time as fecal progestagen concentration increased (in line with P2). There were a few identifiable peaks in

the daily sum of activity during the fecal sampling period, though larger peaks were observed afterwards (P3). However, when exploring both T_v and activity registrations which occurred after the fecal sampling period, a periodic peak in activity was found in close relation to when T_v increased (P2 and P3). The interval of this pattern (about 27 days) aligns well with the expected duration of the estrous cycle in moose [17]. Moreover, we found that the interval length between the first two T_v patterns (between September and October) was significantly shorter than the following two (between October and November). If the interval between observed T_v patterns reflects the length of the estrous cycle in moose, as it is reported for cattle [77], it is possible that the initial estrous cycle of a breeding season in moose may be shorter than the following ones, as has been seen in other seasonal breeders [78–80].

A possible explanation behind fewer observed patterns in activity is that accelerometry values as a measure of estrous behavior is presumably less appropriate for a free-ranging species like moose (captive or wild) compared to domestic cattle which are often kept in a more confined setting during estrous monitoring. Furthermore, the activity values included in our model were summed to daily values, meaning that higher resolution variations within a day were lost, and since estrus is limited to a short interval (1–36 h [17]), using a mean daily value could reduce the representability of estrus-associated variations. These uncertainties made it challenging to include collar activity data in a statistical model and interpret the results. To increase the interpretability and application of collar activity, future studies should investigate the relationship between accelerometry registrations and specific behavioral observations in moose, similar to what has been done for other species of both domestic [81, 82] and wild ruminants [83, 84].

The high explained deviance (72.30%) of the model with daily mean T_v as a response variable supports that there is a relationship between luteal activity and the identifiable pattern which was observed for T_v (P2). According to our model predictions, the rise in collar activity occurs 18 days after the predicted rise in T_v , and on an individual level each observed activity peak tended to occur following an observed increase in T_v . Although the model with activity as a response variable had a lower explained deviance, this indicates that the elevation in T_v is likely not associated with an increase in activity but rather an increase in progesterone hormone, which is known for its thermogenic effects [85].

Despite a high explained deviance, it appeared difficult to generalize the predictions of our model as some moose exhibited a rise in T_v either before or after OLA (range = 7 days before to 3 days after OLA), whereas our

final model predicted that T_v would rise 5 days before OLA. Delay between production and excretion of fecal progesterone [86], sample collection, subsampling, and assay type are all factors that contributed to this inaccuracy. In summary, even though our study demonstrates patterns in T_v which are associated with luteal activity, this variable should not be utilized as a general predictor for luteal activity in moose without the support of other methods to monitor reproductive physiology and behavior.

In contrast, the explained deviance (39.30%) of the model with collar activity as a response variable along with OLA and total daily precipitation as explanatory variables indicates that activity is a weaker predictor for the initial onset of luteal activity in captive moose during the first estrous cycle of the season (P3). Redden et al. [47] found that T_v and activity monitoring, using pedometers, yielded similar estrus detection rates in cattle (81% and 80%, respectively) and some authors have even found that monitoring mounting activity can identify low-intensity estrous behavior which would otherwise be labeled as “silent ovulation” [87]. However, others have found that monitoring temperature can yield better detection rates than monitoring activity [88, 89], which also seems to be the case in the present study. Perhaps activity would have been a good predictor for the subsequent estrous cycles if we had been able to include data which exceeded the fecal collection period, when significantly higher activity peaks were observed.

Conclusion

This study supports the presence of an identifiable pattern in T_v associated with luteal activity in moose, and that succeeding T_v and activity patterns may be indicative of luteal activity in succeeding estrous cycles. These findings offer a new perspective on physiological variables that may be further explored to answer questions about reproductive phenology in moose and indicate that methods which are applied in domestic ruminants may be applicable to wild ruminants as well. Without further research involving more specific and/or additional combinations of methods to monitor reproductive physiology and behavior, these variables should not yet be utilized as general predictors for luteal activity in moose. Additional studies which include a method for confirmation of estrus, ovulation and/or copulation should be conducted to investigate how these events align with our findings before developing methods to monitor reproduction in wild moose. Including this information would enhance our current knowledge about reproductive characteristics of moose and serve a pivotal role in making well-informed management and conservation decisions for the species.

Abbreviations

GAMM	Generalized additive mixed model
GPS	Global positioning system
LH	Luteinizing hormone
OLA	Onset of luteal activity
T_a	Ambient temperature
T_v	Vaginal temperature
VIT	Vaginal implant transmitter

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40317-023-00345-y>.

Additional file 1: Figure S1. Correlations between onset of luteal activity (OLA) and other explanatory variables. Correlations between the explanatory variable onset of luteal activity (OLA) and ordinal day, daily ambient temperature (°C) and daily vapor pressure (hPa) 0.96, -0.77 and -0.71, respectively). Blue dots and line = locally estimated scatterplot smoothing line. Gray region around the blue line = 95% confidence interval.

Additional file 2: Figure S2. Correlations between explanatory variables. Correlations between the explanatory variables daily range of ambient temperature (T_a) and solar radiation, and between wind speed and relative humidity (0.74 and -0.63, respectively). Blue line = locally estimated scatterplot smoothing line. Gray region around the blue line = 95% confidence interval.

Additional file 3: Figure S3. Vaginal temperature, collar activity, and fecal progesterone (2). Graphs from two captive female moose that were 9 years (IDs = 44494 and 44495) containing daily values for mean vaginal temperature (blue line) and daily sum of activity (red line) recorded between July 23rd and November 22nd, 2021, and fecal progesterone concentrations recorded between August 23rd to October 15th, 2021. Dashed vertical blue line = onset of luteal activity, which is on the 25th of September for the top graph and on the 24th of September on the bottom graph. Dashed horizontal red line = individual fecal progesterone threshold.

Additional file 4: Figure S4. Vaginal temperature, collar activity, and fecal progesterone (3). Graphs from two captive female moose that were 12 years (ID = 30683) and 13 years (ID = 30679) containing daily values for mean vaginal temperature (blue line) and daily sum of activity (red line) recorded between July 23rd and November 22nd, 2021, and fecal progesterone concentrations recorded between August 23rd to October 15th, 2021. Dashed vertical blue line = onset of luteal activity, which is on the 25th of September for the top graph and on the 23rd of September on the bottom graph. Dashed horizontal red line = individual fecal progesterone threshold.

Additional file 5: Figure S5. Vaginal temperature, collar activity, and fecal progesterone (4). Graph from an 18-year-old captive female moose (ID = 30682) containing daily values for mean vaginal temperature (blue line) and daily sum of activity (red line) recorded between July 23rd and November 22nd, 2021, and fecal progesterone concentrations recorded between August 23rd to October 15th, 2021. Dashed vertical blue line = onset of luteal activity, which is on the 15th of September for this individual. Dashed horizontal red line = individual fecal progesterone threshold.

Additional file 6: Figure S6. Vaginal temperature, collar activity, and fecal progesterone from excluded animals (1). Graph from a 12-year-old captive female moose (ID = 30680) containing daily values for mean vaginal temperature (blue line) and daily sum of activity (red line) recorded between July 23rd and November 22nd, 2021, and fecal progesterone concentrations recorded between August 23rd to October 15th, 2021. This individual had missing vaginal temperature data from August 12th. Dashed vertical blue line = onset of luteal activity, which is on the 18th of September for this individual. Dashed horizontal red line = individual fecal progesterone threshold.

Additional file 7: Figure S7. Vaginal temperature, collar activity, and fecal progesterone from excluded animals (2). Graphs from two 2-year-old captive female moose (IDs = 44496 and 44497) containing daily values for mean vaginal temperature (blue line) and daily sum of activity (red line) recorded between July 23rd and November 22nd, 2021, and fecal progesterone concentrations recorded between August 23rd to October 15th, 2021. No onset of luteal activity was identified for these individuals. Dashed horizontal red line = individual fecal progesterone threshold.

Additional file 8: Figure S8. Vaginal temperature, collar activity, and fecal progesterone from excluded animals (3). Graphs from two captive female moose that were 2 years (ID = 44498) and 19 years (ID = 30678) containing daily values for mean vaginal temperature (blue line) and daily sum of activity (red line) recorded between July 23rd and November 22nd, 2021, and fecal progesterone concentrations recorded between August 23rd to October 15th, 2021. No onset of luteal activity was identified for these individuals. Dashed horizontal red line = individual fecal progesterone threshold.

Additional file 9: Table S1. Summary of the response and explanatory variables included in the model selection process. **Table S2.** Fecal progesterone classification data from female moose (n = 12) from August to October 2021. **Table S3.** Estimated parameters for explanatory variables in the model with vaginal temperature as a response variable. **Table S4.** Model combinations and ranking with vaginal temperature as the response variable. **Table S5.** Estimated parameters for explanatory variables in the model with activity as a response variable. **Table S6.** Model combinations and ranking with activity as the response variable.

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Author contributions

AE, DT, JC, ER and JH-P contributed to the study design. DT and JC immobilized the study animals, in addition to deploying and retrieving biologging devices. JH-P, DT and JC performed data collection, with data management and statistical analysis done by JH-P, LL, and AT. JH-P prepared the figures and drafted the manuscript. All authors participated in reviewing and approving the submitted version.

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Availability of data and materials

The data sets used and analyzed for this study are available on reasonable request from the Alaska Department of Fish and Game and subject to a data sharing agreement.

Declarations

Ethics approval and consent to participate

All animal care, handling and experimentation procedures at the Kenai Moose Research Center were approved by the Animal Care and Use Committee, Alaska Department of Fish and Game, Division of Wildlife Conservation (protocol number 0086-2020-40).

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no competing interest.

Author details

¹Department of Forestry and Wildlife Management, Faculty of Applied Ecology and Agricultural Sciences, Inland Norway University of Applied Sciences, Anne Evenstads vei 80, 2480 Koppang, Norway. ²Faculty of Veterinary Medicine, Norwegian University of Life Sciences, 1432 Ås, Norway. ³Alaska Department of Fish and Game, Kenai Moose Research Center, 43961 Kalifornsky Beach Road, Suite B, Soldotna, Alaska 99669, USA. ⁴Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden.

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