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# Using kinematics to detect micro-behavioural changes relative to ovulation in naturally cycling tie-stall dairy heifers

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ABSTRACT

Estrus detection rates can be as low as 35% in Canadian tie-stall dairy herds. Low efficacy in tie-stalls is likely due to difficulties in detecting visual signs of estrus because cow movements are more restricted compared with free access stall systems. The overt, obvious behaviours an animal exhibits can be defined as its "macro-" behavioural repertoire, that is, behaviour which can be observed by the naked eye or 'standard' visual observation. In addition, an animal may also display more subtle "micro-" behaviours or "fidgets" which cannot be easily observed or measured through visual observation alone. Three-dimensional kinematics is one way to detect these micro-behavioural changes as kinematics characterizes movement in a very precise way. The objective of this proof of concept study was to investigate, using 3D kinematics, whether tie-stall dairy heifers showed micro-behavioural changes relative to ovulation. Fourteen Holstein heifers were recruited into the study at 43 d in milk and underwent kinematic assessment from 14 d after a first observed ovulation until 2 d after a second ovulation. Kinematic assessment involved placing seven markers on strategic bony landmarks on each heifer's spine and hips. Each heifer was filmed for 15 min daily using six Vicon motion capture cameras as it stood tethered in a tie-stall. The middle 5 min of each 15 min assessment clip was analyzed to control for fidgeting due to novelty of the test environment or boredom. Data for each heifer were standardized to the day of second ovulation. The hip and spine segments for each heifer were reconstructed and labelled digitally using Nexus software (v 2.3). Frequency data were collected for nine behaviours: Macro- (movements > 100 mm), Mid- (40–100 mm), Min- (20–40 mm) and Micro- (10–20 mm) shifts forward and back, and left and right as well as hip tilts. Heifers shifted a distance of 10–40 mm forward and back (Min- $F_{(4,55)} = 5.22$, $P = 0.001$, Micro- $F_{(4,55)} = 5.17$, $P = 0.001$) and side to side (Min- $F_{(4,55)} = 3.69$, $P = 0.01$, Micro- $F_{(4,55)} = 4.92$, $P = 0.002$) and tended to tilt their hips ($F_{(4,55)} = 2.15$, $P = 0.08$) more frequently relative to ovulation. Micro-behaviours were most frequent within a 24 h window before the day of ovulation. This proof of concept study is the first to demonstrate that tie-stall dairy heifers show subtle micro-behavioural fidgets relative to ovulation which can be detected using 3D kinematics.

## 1. Introduction

Reproductive performance is one important aspect of dairy cow management. It relies on the timely and accurate detection of when a cow is sexually receptive – the period known as estrus (Firk et al., 2002). Estrus detection rates can be as low as 35% in Canadian dairy herds regardless of housing system (Ambrose and Colazo, 2007), and pregnancy rates as low as 17.6% (Denis-Robichaud et al., 2016).

The most utilized method of estrus detection in Canada is visual observation of standing to be mounted, with approximately 44% of herds using this method as their only detection technique (Denis-Robichaud et al., 2016). Visual observation alone has highly variable detection rates ranging from 37% (Van Vliet and van Eerdenburg, 1996) to 67% (Van Eerdenburg et al., 1996) and even up to 90% in one particular case (Hall et al., 1959). In addition, visual observation can be labour and time intensive with observations of 30 min up to five times daily needed in order to catch estrus signs and be effective (Mee, 2004; Roelofs et al., 2010; Van Vliet and van Eerdenburg, 1996).

The major shortcoming with the efficacy rates reported above is that all studies have been conducted with cows on pasture or in free-stall systems. In contrast, cows kept in tie-stalls are restricted in their mounting movement compared to free stall cows and have a greater number of sub-estrus or silent estrus events. For example one study observed 15 sub-estruses and 11 silent estruses in tie-stalls compared to four and three events, respectively, on pasture (Palmer et al., 2010). Tie-stall dairies represent 75% of all Canadian dairy herds (Canadian Dairy Information Centre, 2017), therefore a major opportunity exists to develop more effective, feasible and non-invasive estrus detection

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techniques for cows in this type of housing system.

The overt, obvious behaviour an animal exhibits can be defined as its "macro-" behavioural repertoire, that is, behaviour which can be observed by the naked eye or 'standard' visual observation. Examples may be Walking, Mounting or Standing. In contrast, an animal may also display more subtle "micro-" behaviours or "fidgets" which cannot be easily observed or measured through visual observation alone (Bench and Schaefer, 2012). Examples, when standing, may be subtle shifts in weight from one leg to another or forward and back movements to redistribute weight bearing on the feet (Duarte and Zatsiorsky, 1999). Therefore the macro-behaviour, which may indicate that the animal appears to be motionless, can be deconstructed into micro-behavioural components, which indicate the animal is subtly shifting or fidgeting. To our knowledge, there is currently no indication in the published literature whether non-human animals display fidgeting behaviour or in which scenarios.

Three-dimensional kinematics may be a way to detect the presence of micro-behavioural changes. Kinematics characterizes movement in a very precise way by defining specific points on a body and tracking how those points change within a three-dimensional co-ordinate system (Beggs, 1983). This is done by placing markers on the body and recording movement with an optical or stroboscopic camera system. While kinematics has been widely used to detect subtle changes in gait characteristics associated with lameness in dairy cows (Carvalho et al., 2007; Maertens et al., 2011; Pluk et al., 2012; Pluym et al., 2013; Schlageter-Tello et al., 2014; Song et al., 2008; Van Hertem et al., 2014; Van Nuffel et al., 2013), no one has used the technology to investigate other dairy cow health states, such as estrus.

Therefore, the objective of this proof of concept study was to investigate, using 3D kinematics, whether tie-stall dairy heifers show micro-behavioural changes in behaviour during estrus. We hypothesized that, during estrus, heifers would increase the frequency of shifting left and right, representative of shifts in weight over the hind-limbs, and forward and back, representative of a shift in weight from the hind-limbs to the fore-limbs. We also predicted that heifers may tilt their hips forward and back, similar to a subtle, lordosis-type posture.

## 2. Methods

### 2.1. Animals and general care

All procedures were approved by the University of Alberta Animal Care and Use Committee: Livestock (Protocol AUP 00001652). All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009). The study was conducted at the Dairy Research and Technology Centre (DRTC), a tie-stall facility at the University of Alberta, Edmonton, Alberta, Canada from June to October 2016. Fourteen naturally cycling Holstein heifers in their first lactation were used. Heifers were provided access to water and a total mixed ration feed ad libitum. Total mixed ration was formulated according to National Research Council guidelines (National Research Council, 2001) and consisted of silage (barley and alfalfa), grain (barley or corn), hay (alfalfa or grass) and mineral supplements. Heifers were milked in-stall twice daily between 0400 and 0600 and 1530 and 1730.

Because heifers were naturally cycling, each heifer underwent ovarian mapping, at 1500, to track her ovarian cycle and indicate the timeline for kinematic assessment (Fig. 1). Each heifer's ovaries were scanned every second day, starting at 43 d in milk, using ultrasonography to track follicular development and corpus luteum regression. Ovarian mapping continued until the first ovulation assessed in the study period occurred, denoted by the disappearance of the dominant follicle. The day of ovulation represented Day 0 for that individual (Fig. 1). From Day 0 to 7 she received routine care as detailed above with no ovarian mapping. On Day 7 ovarian mapping resumed every second day until Day 14. Between Day 7 and Day 14 the heifer was also habituated to walking from her home stall to the kinematic assessment area (See Section 2.2). From Day 14 until 2 d after her second ovulation assessed in the study period, the heifer underwent daily ovarian mapping and kinematic assessment (See Section 2.4).

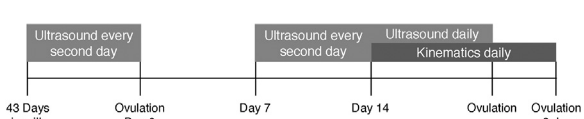


**Fig. 1.** Timeline of experimental procedure.

### 2.2. Heifer habituation

To mitigate any confounding behavioural effects of a novel assessment environment or having markers adhered to their body, heifers were first habituated to stand in a stall designated for kinematic assessment and being touched by the experimenter. The assessment area was an empty stall (approximately 52 in. wide by 70 in. long) in the middle of the dairy barn. The stalls directly on either side of the assessment area were kept clear to minimise potentially confounding movement from interactions with other cows. The remaining stalls in the surrounding area (that is, two stalls away from the assessment area) were occupied by cows. For five out of seven days between Day 7 and Day 14, heifers were trained to go into the kinematic assessment area and stand alone for a period of 10 min. The same two people, the handler and experimenter, handled the heifer during habituation.

The handler guided the heifer from her home stall to the kinematic assessment area. A small feed ration of one handful of hay was provided as a reward to encourage the heifer to enter the novel environment on future occasions. Both the handler and experimenter then stroked the heifer and applied light squeezing pressure to the areas which would later have kinematic markers applied, namely the lower spine and pelvis. This continued for approximately 1 min, after which time the handler and experimenter left the assessment area and waited in a room adjacent to the barn. The handler and experimenter remained out of view of the heifer for 10 min while the heifer habituated to the assessment area. After 10 min the heifer was returned to her home stall.

### 2.3. Kinematic setup

Six kinematic cameras (Bonita, Vicon Motion Systems Ltd., Denver, Colorado) were mounted in a semi-circle above and approximately 2 m behind the assessment stall. The kinematic cameras were connected via Ethernet cables to a Powered Over Ethernet (POE) system (LevelOne GEP-1622, Digital Data Communications GmbH, Germany) which was connected to a desktop computer (Precision Tower 5810, Dell Inc., Toronto, Canada) running Vicon Nexus 2.3 software. The computer system was on a movable audio visual trolley, which was positioned in front, but out of view, of the heifer in the assessment area. The kinematic cameras were set to record at 100 fps.

The kinematic assessment area was calibrated daily before kinematic testing began. Calibration was achieved using a Vicon Active Wand (2.0, Vicon Motion Systems Ltd., Denver, Colorado) together with the Calibration and Set Volume Origin pipelines in the Nexus program.

A labelling skeleton was created using a model cow not included in the study. Seven reflective kinematic markers (Life Science Basic Kit, Vicon Motion Systems Ltd., Denver, Colorado) were adhered temporarily on bony landmarks on the spine and hips using four small, 20 mm × 20 mm, squares of Tuck Tape™ (Fig. 2A). For this proof of concept study, it was decided to first focus on the hip and spine movements based on the fact that standing to be mounted is the most commonly-utilized detection posture for dairy cows (Nordéus et al., 2012), so any micro-behavioural movements are likely to be observed in the hip or spine regions. A small (9.5 mm) marker was attached to each location on the lumbar spine, caudal spine, sacral spine (three markers total). A