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Vocalization as an indicator of estrus climax in Holstein heifers during natural estrus and superovulation

Volker Röttgen,*† Frank Becker,† Armin Tuchscherer,‡ Christine Wrenzycki,§ Sandra Düpjan,* Peter C. Schön,* and Birger Puppe*#¹

*Institute of Behavioural Physiology,

†Institute of Reproductive Biology,

‡Institute of Genetics and Biometry, Leibniz Institute for Farm Animal Biology (FBN), D-18196 Dummerstorf, Germany

§Chair of Molecular Reproductive Medicine, Clinic for Veterinary Obstetrics, Gynecology and Andrology, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, D-35392 Giessen, Germany

#Behavioural Sciences, Faculty of Agricultural and Environmental Sciences, University of Rostock, D-18059 Rostock, Germany

ABSTRACT

The reliable detection of estrus is an important scientific and practical challenge in dairy cattle farming. Female vocalization may indicate reproductive status, and preliminary evidence suggests that this information can be used to detect estrus in dairy cattle. The aim of this study was to associate the changes in the vocalization rate of dairy heifers with behavioral estrus indicators as well as test the influence of the type of estrus (natural estrus vs. superovulation-induced estrus). We analyzed 6 predefined estrus-related behavior patterns (standing to be mounted, head-side mounting, active mounting, chin resting, being mounted while not standing, and active sniffing in the anogenital region) and vocalization rates in the peri-estrus period (day of estrus \pm 1 d) of 12 German Holstein heifers using audio-visual recordings. Each heifer was observed under natural estrus and a consecutive superovulation induced by FSH and cloprostenol. Estrus was determined by behavioral patterns and confirmed by clinical examination (vaginocopy and ultrasound imaging of the ovaries) as well as by the concentration of peripheral progesterone. Estrus behavior and vocalization rates were analyzed in 3-h intervals (an average of 19 intervals for each heifer), and an estrus score was calculated based on the 6 behaviors. The interval with the highest estrus score (I₀) was considered the estrus climax. We demonstrated similar time courses for the estrus score and vocalization rate independent of estrus type. However, in natural estrus, the maximum vocalization rate (\pm SE) occurred in the interval before estrus climax (I₋₁; 42.58 ± 21.89) and was significantly higher than that in any other interval except estrus climax (I₀; 27.58 ± 9.76). During natural

estrus, the vocalization rate was significantly higher within the interval before estrus climax (I₋₁; 42.58 ± 21.89 vs. 11.58 ± 5.51) than under superovulation. The results underscore the potential use of vocalization rate as a suitable indicator of estrus climax in automated estrus detection devices. Further studies and technical development are required to record and process individual vocalization rates.

Key words: estrus detection, estrus behavior, bioacoustics, cattle

INTRODUCTION

Estrus detection is critical for reproductive performance and therefore of great concern in the dairy cattle industry. Effectively identifying cows in estrus is one of the everyday challenges faced by dairy farmers.

Visual detection of estrus remains one of the most reliable methods of identification, but it involves high staff costs (Rae et al., 1999; Rorie et al., 2002; Rutten et al., 2014) because the herd must be observed up to 3 times daily for 20 to 30 min. VanVliet and VanEerdenburg (1996) introduced an estrus scoring system based on behavioral observations, in which different behaviors are weighed according to their specificity to indicate estrus (Table 1) and then aggregated into a single score. The need for a reliable but more cost-effective method to identify the optimal insemination time for dairy cattle has promoted the development of automatic estrus detection devices. Typically, such devices only use one parameter to detect estrus, but some authors suggest that combinations of parameters could be more effective (Reith and Hoy, 2012). The following combinations of indicators have been proven to increase estrus detection rates: (1) visual estrus detection, activity measurements, and mounting detection (Peralta et al., 2005); (2) visual estrus detection with either a hormone-treated steer or tail paint and activity measurements combined with milk data (yield,

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¹Corresponding author: puppe@fbn-dummerstorf.de

Table 1. The estrus behaviors and their definition¹

Behavior	Definition	Estrus score points
Standing	Standing to be mounted: accepting the mounting of another heifer.	100
Mounting head	Mounting with the claws above the carpal joint level.	45
Mounting	Focal subject trying to mount a herd member from behind with both front legs above the height of gambrel level.	35
Chin rest	The chin of the observed heifer resting on the dorsal line of a herd member, between the withers and the tail, and not more than 10 cm along the back line.	15
Mounted run	Animal moving away from the mounting attempt of a group member.	10
Sniffing	The nose of the focus animal coming close to the pudendal region of the group member.	10

¹The estrus score points are the factors that were multiplied by the recorded count of the behavior per interval (according to Van Vliet and VanEerdenburg, 1996).

temperature, and conductivity; Firk et al., 2002); and (3) activity, rumination time, and BW (Reith and Hoy, 2012). The efficacy and accuracy of automated estrus detection devices might also be increased by incorporating additional, yet unstudied parameters that are directly or indirectly related to estrus or estrus behavior. Vocalization rate is one such behavioral trait with promising characteristics for estrus detection (Schön et al., 2007; Dreschel et al., 2014), but no studies have described a time course for vocalization rates relative to established behavioral signs of estrus in dairy cows.

Changes in female vocalization that are dependent on the sexual cycle have been almost completely neglected in behavioral and bio-acoustic research. Some data are available from studies of humans (Bryant and Haselton, 2009), mammals (Charlton et al., 2010), birds (Langmore and Davies, 1997; Langmore et al., 1996), and amphibians (Tobias et al., 1998), and these studies describe different functions of female vocalizations that contribute to specific mating behaviors. In some species, vocalizations or specific elements of the vocal repertoire increase during estrus [e.g., African elephant (*Loxodonta africana*), Leong et al., 2003; Long-Evans rats (*Rattus norvegicus*), Matochik et al., 1992].

In cattle, vocalization alterations can occur in the context of pain (Stookey et al., 1996) or handling (Grandin, 2001), and some investigators have decoded the information conveyed by specific vocalizations (e.g., individual recognition, delayed milking, or hunger; Ikeda and Ishii, 2008; Jahns, 2008). In previous studies, we demonstrated that vocalization rates increase in heifers in estrus, both in tiestalls and group housing (Schön et al., 2007; Dreschel et al., 2014). However, none of these studies have demonstrated the temporal course of the vocalization rate in relation to estrus behavior, which is necessary for the development of automated estrus detection systems.

In cattle breeding, different hormonal treatments are commonly used to enhance fertility and breeding progress as well as reduce the number of nonpregnant

days. Superovulation is a hormonal treatment that affects ovarian function by increasing the number of recruited primordial follicles and growth of dominant follicles, and it changes the number of ovulations and the concentrations of peripheral hormones (Roberge et al., 1995). Moreover, evidence indicates that superovulation not only increases the serum estradiol level but estrus behavior as well (Jeziarski, 1993; Jimenez et al., 2011). Superovulation in cattle can be achieved by the administration of FSH in a descending dosage and induced luteolysis via PGF_{2α}.

The primary aim of our study was to characterize the variation in vocalization during estrus in relation to the behaviors that are typically used for visual estrus detection, and we also analyzed the temporal association between ovulation and vocalization. The secondary aim of this study was to explore if the change in estrus behavior under superovulation treatment also affects the temporal vocalization pattern.

MATERIALS AND METHODS

In this study, we examined 12 German Holstein heifers in 3 groups of 4 animals each during natural estrus (NAT) and a subsequent superovulation treatment (SUP). The animals were housed in the experimental pen on the grounds of the Experimental Facility for Cattle at the Leibniz Institute of Farm Animal Biology, Dummerstorf, Germany. The experimental pen was a solitary building that was partially sound insulated, meaning that environmental noise was reduced and sound reflections within the pen were minimized by sound-dampening wall padding. Each group was continuously housed in the pen throughout the experimental period of approximately 60 d between March and November. The 5 × 10 m pen was equipped with rubber flooring and wood shavings as bedding material, 3 drinking spots, and a self-locking feed barrier.

The average age of the heifers was 15.6 mo (range of 13.5–17.6 mo). Before being entered into the study,

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