



# Automatic oestrus detection system enables monitoring of sexual behaviour in sheep



M. Alhamada<sup>a</sup>, N. Debus<sup>a</sup>, A. Lurette<sup>a</sup>, F. Bocquier<sup>a,b,\*</sup>

<sup>a</sup> INRA, UMR868 Systèmes d'élevage méditerranéens et tropicaux, F-34000 Montpellier, France

<sup>b</sup> Montpellier SupAgro, Dept. MPRS, F-34000 Montpellier, France

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## ABSTRACT

An experiment was conducted to observe the oestrous behaviour in ewes through an automated oestrus detection system. Five mature adult Merino male rams, equipped with an electronic detector (Alpha-D), were included with a total of 60 ewes from the same breed. The libido of the males was assessed as the number and frequency of mounts. The accepted mountings by ewes were recorded by an electronic identifier worn by the female. In the first synchronized oestrus (C1), the ewes were exposed to rams wearing an anti-mating apron. In the following spontaneous cycle (C2), all the rams were allowed to mate. In the third cycle (C3) and the fourth cycle (C4), the rams were assumed to detect ewes in oestrus (open ewes). After the exclusion of isolated readings, the mean interval from sponge withdrawal to onset of oestrus was  $31 \pm 11$  h and duration of oestrus in C1–C3 was  $22 \pm 18$ ,  $16 \pm 11$  and  $16 \pm 8$  h, respectively. The libido differed widely among the rams and cycles number with an average number of mounts per ram in C1–C4, that were 213, 322, 85 and 3 respectively. It is concluded that all the females were correctly detected and the assessed libido of males was repeated.

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## 1. Introduction

The key to the success of fertilization, whether natural or after artificial insemination, depends on identifying the beginning and the duration of oestrus. This critical period may last from several hours to several days. Even after hormonal treatment, extensive synchronization of oestrus has not yet been achieved. Synchronization is critical when attempting to inseminate ewes without hormonal treatment (e.g. on organic farms).

The ram reacts sexually when a ewe responds to his advances by becoming immobilized. The ability of rams to serve large number of ewes in a short time is of great importance for the fertility of the flock as a whole (Alexander et al., 2012). The ram's libido should be another key to the reproductive success. However up to now routine evaluation of males' libido has been limited by the time, labour and facilities required (Alexander et al., 2012). Since Price et al. (1992) demonstrated that the frequency of mounting is a reliable estimate of the libido and of the mating success of sexually experienced rams; the ability to quantitatively and automatically

evaluate rams based on this criterion would certainly be a major advance.

The traditional way of detecting oestrus in sheep is visual observation of a ram searching for receptive ewes. This can be achieved by tight direct control of the ram's activity either intermittently (at intervals of from 6 to 12 h; Bathaei, 1996; Martemucci and D'Alessandro, 2011; Fabre-Nys and Venier, 1987) or by checking for crayon marks left on the fleece of the ewes by rams equipped with a harness (Romano et al., 2001). Both methods are time consuming and limited to research, since observations have to be made night and day for at least one complete cycle (i.e. 17 days) before a decision can be made on whether the ewes had an oestrus or not. On the farm, the only solution is to periodically observe mating behaviour, which is very time consuming. In this case, the main limitations are many errors due to poor identification of animals plus the difficulty in counting the number of times each ewe is mounted. Furthermore, as frequently observed by shepherds, some rams do not necessarily exhibit normal sexual behaviour in the presence of humans (Maina and Katz, 1999).

Although the mating behaviour of sheep has been studied for many years (Banks, 1964), to our knowledge, this is the first time that mating behaviour and the kinetics of the occurrence of oestrus in ewes is reported an automatic device, Alpha Detector (Alpha-D), which records all mountings by the rams in a flock. This detector, which has already been the subject of a preliminary evaluation

\* Corresponding author at: Montpellier SupAgro, UMR SELMET, 2 place P. Viala, Bât 22, F-34060, Montpellier Cedex 1, France.

E-mail address: [bocquier@supagro.fr](mailto:bocquier@supagro.fr) (F. Bocquier).

(Alhamada et al., 2016), ensures 94% identification of each mount done on a ewe in standing oestrus.

In addition to the importance of detecting oestrus for the successful management of a profitable farm, early pregnancy diagnosis in sheep is extremely important because of the seasonal breeding of this species. Failing to breed corresponds to variable delays in lambing in a certain period or season. Many means of detecting pregnancy are available for domestic ruminants, the simplest being detection of open ewes after breeding (Ishwar, 1995). However, an efficient automated device for the detection of non-returning ewes into oestrus would be far easier than manipulating ultrasonic devices or dosing pregnancy specific proteins like ovine pregnancy specific protein B (oPSPB).

The aim of this study was to quantify and analyse three different aspects of an automated oestrus detection system. First, the quantitative evaluation of the kinetics of the occurrence of oestrus after hormone treatment and during the following cycles. Second, the assessment of the libido of males through the number and frequency of mounts. Third, the assessment of the interest of oestrus detection as a pregnancy test. The overall aim of the experiment was to establish benchmarks for on-farm use of the device.

## 2. Materials and methods

All the experiments were approved by INRA and Regional Ethics committee in Montpellier, France and complied with the 1985 French Animal Research Act in accordance with ethical principles laid down in the European Union directive 2010/63/EU.

### 2.1. Animals and experimental design

The experiment was conducted at the Fréjorgues Experimental Station (Lat. 43°59'N, Long. 4°39'E) in south-eastern France during the breeding season from the 17th of October to the 15th of December. Sixty multipara Arles Merino ewes born through the same year (3.6 years of age) with a mean body weight (BW) of  $59.7 \pm 5.8$  kg, and a mean body condition score (BCS; scale 1–5, 1 = emaciated, 5 = obese) of  $2.9 \pm 0.1$  were used in this study. Oestrous cycles were synchronized in all ewes by the insertion of an intravaginal sponge impregnated with 20 mg fluorogestone acetate (FGA) (Chrongest<sup>®</sup> CR, Intervet, Angers, France). After 14 days, the sponge was removed and each ewe received an intramuscular injection of 400 IU of equine Chorionic Gonadotropin (eCG; SYN-CRO PARTI<sup>®</sup> PMSG, Ceva Santé Animale, Libourne, France). The ewes were monitored during the four succeeding cycles by the device. Five fertile 3–4-year-old adult rams of the same breed with a BW of  $80.5 \pm 3.8$  kg were used to detect oestrus in a unique group of 60 ewes throughout the four oestrous cycles (which normally last 17 days). These rams were introduced into the group of females as soon as the sponge was removed (Fig. 1). Females were identified by a transponder glued on their tail (see below). All the rams were equipped with an electronic oestrus detector. During the first cycle (C1), the rams wore aprons to prevent them from mating the ewes. Ewes and rams were kept together for five days (C1) and then separated. Ten days later, the rams were again introduced into the group of ewes with no anti-mating apron and allowed to mate for seven days (C2). The same males were mixed with the females during subsequent natural cycles (C3 and C4) in exactly the same way to identify open ewes and determine the pregnancy rate.

### 2.2. Principles of the oestrus detector

The Alpha-Detector (Alpha-D) used in this experiment had previously been shown to detect 100% of ewes in oestrus, and to detect individual mounts with a 94% accuracy for ewes in standing oestrus, thus enabling the precise measurement of the behaviour

of both males and females (Alhamada et al., 2016). This automated heat detection device (Alpha-Detector<sup>®</sup>, Wallace, Cardet, France) is based on a standalone special reader worn by the male. The reader records the information on the electronic identifier worn by the female accepting mountings. The detector, located in a leather pouch, is held between the front legs of the male by a harness. When the ram mounts, a pressure sensor triggers the reading of the transponder attached to the tail of the female. At each reading, the identification of the female, and the exact date and time are recorded. These data are stored and then transferred to a hand-held device called Alpha-Receptor on request (Alpha-R<sup>®</sup>, Wallace, Cardet, France). The shepherd can select an Alpha-D on the Receptor which scans and find the right Alpha-D. After connection is established, data are transferred remotely ( $\leq 100$  m) to the Receptor by radio signal. The information is extracted and saved on a computer until data processing and analysis. The battery life of the Alpha-Detector usually lasts more than a week depending on number of readings made by rams. Except for recharging battery no manipulation of rams are necessary. The female transponders are Half Duplex (HDX) Tiris<sup>®</sup> Glass Transponders ( $32.0 \times 3.8$  mm; model RI-TRP-WR2B; GIOTEX, Barcelona, Spain) which comply with the 11784/11785 ISO standard for animal identification.

### 2.3. Pregnancy tests

#### 2.3.1. Blood sampling method

Ovine pregnancy-specific protein B (oPSPB) becomes detectable 19 days after conception and increases steadily up to day 30 (Wallace et al., 1997). Blood samples were collected from all ewes on day 49 after the sponge was removed (C1). Ewes were bled at 8:00 am just before their daily meal by jugular venipuncture using vacuum heparinized tubes (Terumo, Sodap, Montpellier, France). Following blood collection, the tubes were centrifuged at 3600 rpm for 20 min at 4 °C, plasma was harvested then stored at  $-20$  °C until assayed for oPSPB. Ovine PSPB concentrations were determined in duplicate with a heterologous radioimmunoassay (RIA) using bovine PSP60 as standard and tracer (Mialon et al., 1993). The sensitivity of the assay was 0.2 ng/ml plasma and intra- and inter-assay coefficients of variation were 6% and 12%, respectively.

#### 2.3.2. Ultrasound method

The ewes underwent transabdominal ultrasonography. Pregnancy diagnosis was performed by a trained operator with an ultrasound scanner (Ovi-Scan; BCF Technology Ltd, Livingston EH54 8TE, Scotland, UK) on day 45 on mated ewes (i.e. post C2). During ultrasonography the ewes were restrained in the standing position in a cage to limit their movements.

#### 2.3.3. Non-return method

Identification of ewes in oestrus during the C3 and C4 cycle also allowed the identification of non-pregnant ewes. Otherwise ewes were considered to be pregnant.

### 2.4. Statistical analysis

As most of the data consisted of a number of readings, frequency data were examined by Chi-square analyses to detect percentage differences among the number of rams mounts. Results are presented as means  $\pm$  SD. The daily mounting activity of rams during highest period of activity of rams was analysed by ANOVA and Tukey test (R Core Team, 2015) using a random effect of Rams (1–5), a Cycle effect (C1 or C2) and effect of number Days in oestrus (day 1–3) nested in Cycle and random error.

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