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Short Communication

Relationship between electrical resistance of cervical mucus and ovarian steroid concentration at the time of artificial insemination in ewes

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ABSTRACT

The purpose of this study was to investigate whether fertile or non-fertile inseminations (AI) in synchronized ewes are correlated with the electrical resistance of cervical mucus (ERCM) and the ovarian steroid concentration. AIs were performed either at fixed-time (group A) or after estrus detection (group B). Retrospective analysis revealed that at AI, pregnant ewes had lower ERCM values and progesterone concentrations than non-pregnant ones ($p < 0.05$). It appears that ERCM may be used as an additional index for fertility enhancement of inseminated ewes.

1. Introduction

Estrus detection is of paramount importance for sustaining high pregnancy rates after estrus synchronization. As opposed to cattle for which a number of commercial methods are available to assist estrus detection [1], in sheep only "teaser" (apron-fitted or vasectomized) rams can be used for this purpose. Under field conditions, estrus detection with teaser animals may compromise pregnancy rates, possibly due to the late detection of ewes that are eligible to be inseminated [2]. Moreover, the presence of males has been shown to alter physiological estradiol concentrations. For the aforementioned reasons and for practicality, fixed-time cervical insemination (AI) is usually performed in estrus-synchronized ewes. Hence, a convenient alternative method for the accurate estrus detection of synchronized ewes would be of great interest for the sheep industry.

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While variations of electrical resistance of cervical mucus (ERCM) have been extensively studied in cows and sows [3], the relevant information for sheep is rather scarce. In cows there is a close relationship between vaginal electrical resistance (VER), progesterone (P4) concentrations and manifestation of estrus behavior [4]; similarly in buffaloes, VER has been found to be associated to P4 concentration [5]. It has been shown that in ewes the lowest impedance of the vaginal mucus occurs prior to estrus, and it remains low for the next 24–48 h [6]. VER is also affected by changes of estradiol to progesterone ratio, or by declining or steadily low P4 concentrations [6]. Assessment of VER before AI may serve as a practical alternative method of estrus detection, or when rams are not available. Thus, in order to improve pregnancy rates, it is prerequisite to accurately select ewes eligible for insemination by using a simple index. In the present study, by taking only one ERCM measurement, we sought to find the most appropriate time for cervical AI; this approach could help the procedure be easily adapted for veterinary practice.

2. Materials and methods

The study was conducted during the breeding season at the Agricultural Research Station of Halkidiki – Northern Greece. Chios ewes (30–42 months; 65 ± 5.5 kg) were kept in a sheltered barn with free access to natural pasture and water. All ewes were at second or third lactation and they had lambed 2–3 months before the onset of the experiment. Estrus synchronization was conducted by a combination of medroxyprogesterone acetate impregnated intravaginal sponges (MAP; 60 mg, Veramix, PROVET, Athens, Greece) and eCG (Intergonan, Intervet, Boxmeer the Netherlands). Sponges stayed *in situ* for 14 days and, at removal, 400 IU eCG, i.m., was administered. In all cases ewes were inseminated with pooled fresh diluted semen from six Chios rams of known fertility at a dose of 200×10^6 spermatozoa. Semen was maintained at 5 °C and AIs were performed 2 h after semen collection. Fifty-three hours after sponge removal, fixed-time cervical AI was performed in ewes of group A ($n = 85$). In group B ($n = 35$), starting 12 h after sponge removal and for the next three days, estrus detection was carried out twice daily using two apron-fitted rams. Any ewe seen standing to be mounted by the ram was considered as being in estrus and AI was performed. All ewes of group A and 21 ewes of group B were inseminated on the same day, while 14 ewes of group B (estrus detected on day 3) were inseminated one day later.

At the time of AI, prior to semen deposition, ERCM was measured by a digital heat detector (Cyclus, A.S. Lima, Sandnes, Norway). The device, destined for use in cattle, had a shaft length of 45 cm, maximum probe diameter of 2 cm, and a probe length of 5 cm. The probe was inserted into the vagina until resistance was felt, indicating contact with the posterior cervix. Three consecutive readings of ERCM were taken before the probe was withdrawn, and the mean value was recorded. At the same time a blood sample was collected from the jugular vein (Venoject, Terumo, Belgium). The blood was left to clot, and the serum was separated by centrifugation ($1100 \times g$; 20 min; 4 °C) and stored at −20 °C until assayed. After extraction, serum concentrations of estradiol-17β (E2) and P4 were determined by radioimmunoassay (RIA) [7]. The radiolabeled E2 and P4 were provided by Amersham Biotech (Buckinghamshire, UK). Assay sensitivities were 3.9 pg/ml and 0.019 ng/ml, intra-assay coefficients of variation were 2.0% and 1.8%, inter-assay coefficients of variation were 7.5% and 6.4%, and recovery rates were 94.4% and 96.2% for E2 and P4, respectively. Thirty-nine to 40 days after AI, pregnancy diagnosis was carried out by trans-abdominal ultrasonography (SonoVet 2000; 4.5–6 MHz convex transducer, Medison CO, Seoul, Korea).

Pearson's chi-square test was used to compare pregnancy rates (%) between groups A and B. Between groups A and B, and between pregnant and non-pregnant ewes, Student's *t*-test (independent) was used to compare serum E2 and P4 concentrations, as well as ERCM values. Linear regression analysis was performed to determine relationships between ERCM values and P4 or E2 concentrations. Statistical analysis was performed using SPSS® 15.0 for Windows (SPSS Inc., Chicago, IL, USA) and probability of $p < 0.05$ was the minimum level of significance.

3. Results and discussion

In ewes, fixed-time AI is usually performed after estrus synchronization but pregnancy rates are not always satisfactory. Based on data from other species [3,5], we selected to examine if ERCM measurement could serve as reliable tool to assess optimal time for AI in order to improve pregnancy rate in ewes. It has been shown that after pharmaceutical estrus synchronization in goats, onset of estrus varies considerably (24–120 h) [8]; hence ERCM measurement may assist in predicting onset of estrus or in verifying that AI is performed at the appropriate time.

The instrument used for the ERCM measurement in the present study was not designed for ewes. However, the probe diameter was similar to that reported by Bartlewski et al. [6] who used a device designed and validated for use in sheep. In our study, pregnant ewes had a mean ERCM value at AI $< 340\ \Omega$ that was significantly lower compared to that of non-pregnant ones (Table 1). In ewes, a close temporal relationship between the decrease of vaginal mucus impedance and manifestations of behavioral estrus was previously reported [6]. However, when MAP is used for estrus synchronization, the characteristic fluctuations of physiological cervical mucus are seriously distorted, and as a result cervical mucus volume, crystallization and spinnbarkeit are increased [9].

After sponge removal in group B, estrus was detected on day 2 in 21 ewes and on day 3 in 14 ewes. The pregnancy rate was lower ($p < 0.001$) in group A (32.9%) compared to group B (65.7%). Progesterone concentration and ERCM values were significantly lower ($p < 0.05$ and $p < 0.001$ respectively) in pregnant compared to not pregnant ewes of both groups (Table 1). Linear regression analysis of data revealed a positive ($p < 0.05$) relation between ERCM and serum P4 in both groups, but not between ERCM and E2. It has been reported that progesterone reduces oviductal epithelial ciliary beat frequency in humans [10]. In addition, high P4 concentrations can affect sperm capacitation in boars [11] and acrosome reaction