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SHORT COMMUNICATIONS

Vaginal cytology and cervical mucus as tools to predict ovulation time in small ruminants

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Abstract
The possibility of using cervical mucus and vaginal cytology as tools to predict ovulation time was assessed in 11 ewes and 11 does raised under tropical conditions. Every 12 h from progesterone removal to ovulation, estrus behavior, cervical mucus, vaginal cytology, and ovarian ultrasound exams were performed. In goats, vaginal cytology had 88% of accuracy on detecting the ovulation time. However, in sheep, there was no cell pattern in the vaginal cytology and cervical mucus varied at ovulation. In conclusion, both vaginal cytology and mucus evaluation may be useful tools to determine the ovulation time in goats; however, both strategies are less accurate in sheep.

Keywords Goat · Ovulatory timing · Sheep · Ultrasound · Vaginal cells

Introduction

A crucial prerequisite for the success of any production system is that animals must have efficient reproductive performance. In general, the use of reproductive biotechniques requires the employment of ultrasound (US) for different purposes. The transrectal US makes possible to assess the animal reproductive stage, identifying ovulation in real time (Haibel 1990). However, since it requires relatively expensive equipment and properly trained people, US is not constantly accessible to the producer. Thus, it is important to study simpler strategies that could be markers for decision making. Parameters associated with ovulation time such as cervical mucus in goats (Fonseca et al. 2017) are a clear example and are often applied to determine the best time to perform artificial insemination (AI). However, the association of cervical mucus and ovulation time in sheep is yet to be demonstrated.

Vaginal cytology provides essential information about the ovulation time in bitches (Schutte 1967). This tool gives valuable data about the phenomena that, due to hormonal changes, occurs throughout the estrous cycle in sheep (Zohara et al. 2014) and goats (Ribeiro et al. 2019). Although the use of vaginal cytology specifically to detect ovulation time is frequently used in some species, it has never been assessed in small ruminants. Thus, we hypothesized that the association of cervical mucus and vaginal cytology may predict with confidence the ovulation time in goats and sheep in a simpler and cheaper way than US. This study assessed the possibility of using cervical mucus and vaginal cytology as tools to predict ovulation time in small ruminants raised under tropical conditions.

Materials and methods

The study was conducted at the non-breeding season, in Coronel Pacheco (21°35′S and 43°15′W), Brazil.

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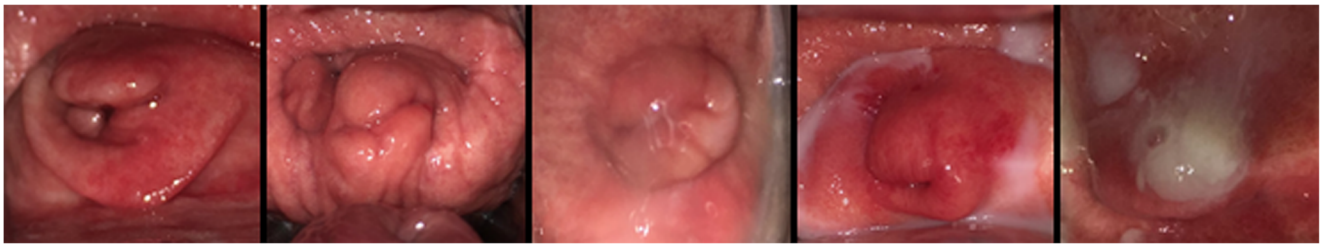

Fig. 1 Cervical mucus types of Santa Inês ewes hormonally induced to estrus. Mucus was assessed from device removal to ovulation and classified as (from the left to right) 1 – crystalline (mucus completely translucent), 2 – crystalline/striated (mucus presents some opacity but not stretch marks), 3 – striated (evident stretch marks within crystalline areas), 4 – striated/caseous (stretch marks coalesce and no visible translucent areas), and 5 – caseous (mucus appears a caseous mass with evident flocculation)

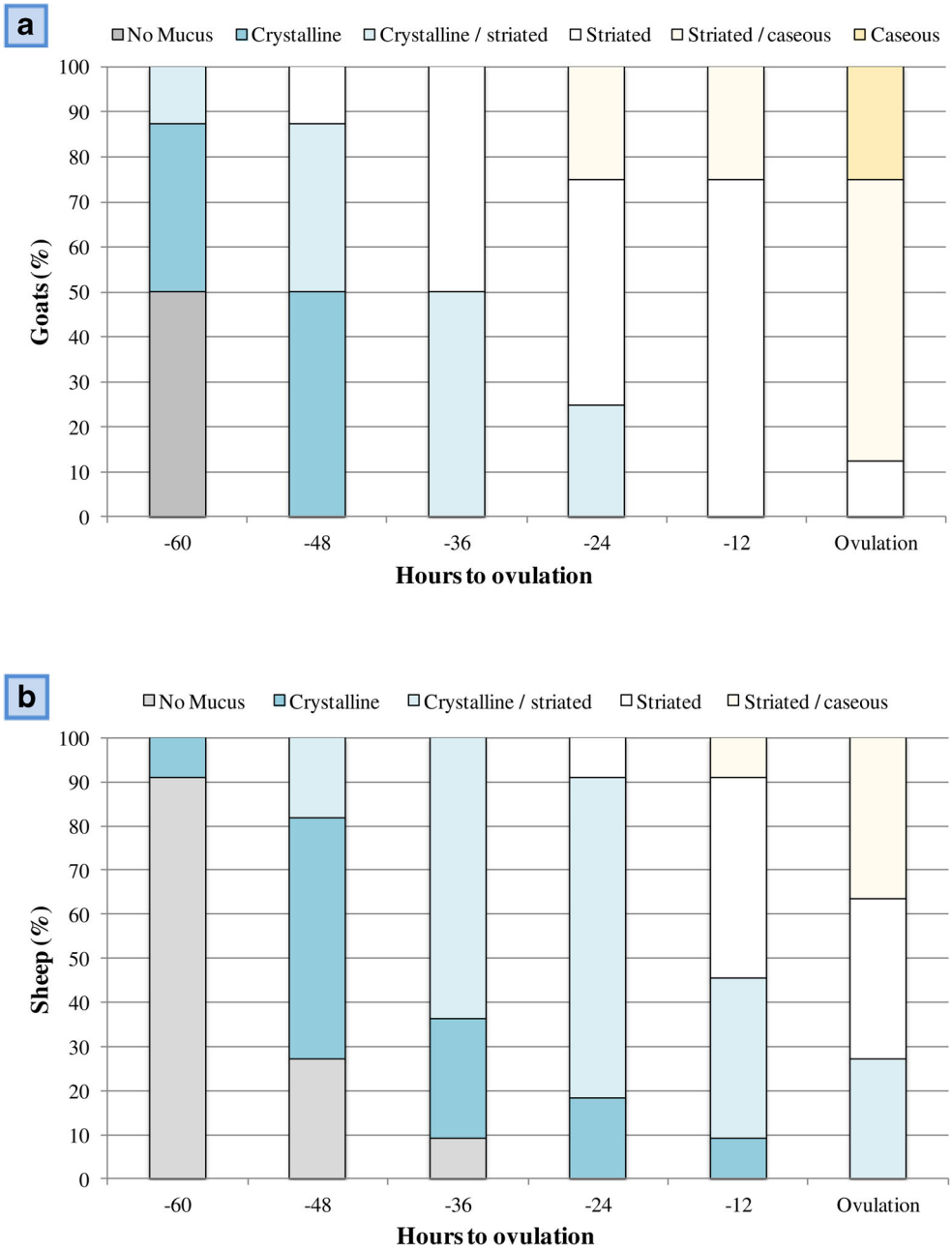

Fig. 2 Cervical mucus type evolution according to the ovulation time in goats and sheep hormonally induced to estrus

Pluriparous Alpine goats (n = 11) and Santa Inês sheep (n = 11), aging 3 years old, with 57 ± 15 kg and 53 ± 10 kg of body weight and 3.5 ± 0.5 and 3.2 ± 0.6 of body condition score (1–5 scale), respectively, were used. All animals were kept in an intensive system and fed corn silage. A balanced concentrate supplement was provided. Mineralized salt and drinking water were available ad libitum. All animals received intravaginal devices containing 0.3 g progesterone (Eazi-Breed CIDR®, Pfizer Animal Health, São Paulo, Brazil) for 6 days, plus 30 μg d-cloprostenol (Prolise®, Syntex, Buenos Aires, Argentina) IV and 200 IU eCG (Novormon® 5000, Syntex) IM 24 h before device removal. Estrus was monitored twice daily aided by fertile bucks and rams.

Cervical mucus, smear collection, and US exams were assessed every 12 h from device removal to ovulation (or up to 96 h). Sterilized Collin vaginal specula were used to classify the mucus flowing from the cervical ostium: 1 – crystalline, 2 – crystalline/striated, 3 – striated, 4 – striated/caseous, and 5 – caseous (Fonseca et al. 2017). A 15-cm-long cotton-tipped sterile swab was inserted into the vagina to a depth of about 5–7 cm. The swab was removed, rolled on a clean glass slide, and stained with Quick Panotic dye (Laborclin Ltda, São Paulo, Brazil). Epithelial cells were classified under a light microscope into parabasal, intermediate, nuclear, and anuclear superficial cells (Schutte 1967). A total of 100 cells were counted under 400X magnification (BX4 microscope, Olympus Corporation, Tokyo, Japan). Transrectal US exams were conducted with a B-mode scanner (7.5 MHz; Mindray®, M5 Vet, Mainland, China). Ovulation time was defined as the day when the largest follicle, previously identified, was no longer detected (Souza et al. 2011).

The possibility of applying both tools was assessed detecting animals, either: true positive (TP), negative (TN), false positive (FP), and negative (FN). Then, calculations were performed: sensitivity [TP/(TP + FN)], specificity [TN/(FP + TN)], positive [PPV = TP/(TP + FP)]) and negative [NPV = TN/(FN + TN)]) predictive values, and accuracy [ACC = (TP + TN/n)]. Nonparametric data were analyzed by McNemar, Kruskal Wallis, and Dunn test, while parametric data were compared by ANOVA and t test. Analyses were performed with available program (BioEstat 5.0, Belém, PA, Brazil) (Ayres et al. 2007) and the confidence level was 5%.

Results

Estrus response rate was 81.8% (9/11) for goats and 90.9% (10/11) for sheep and all these animals ovulated. The interval from device removal to estrus, estrus duration, and interval from device removal to ovulation were, respectively, for goats: 29.3 ± 2.1, 20.0 ± 2.0 h, and 62.7 ± 1.8 h, and for sheep: 34.8 ± 2.1, 15.6 ± 2.7, and 61.2 ± 1.2 h. The cervical mucus type classification in sheep is illustrated in Fig. 1. The cervical mucus evolution through time in both species is shown in Fig. 2. In goats, the median found at the ovulation time was characteristic, indicating a mucus type 4 and goats predominantly ovulated when mucus was 4 or 5 ($P < 0.05$). In sheep, there was no predominance of any mucus type at the ovulation ($P < 0.05$). The possibility of applying the mucus evolution (type 4–5 in goats and type 4 in sheep) as a tool to predict the ovulation time is described in Table 1. In sheep, when including the mucus type 3 (besides type 4), the accuracy was 84.8%

Table 1 Performance parameters* of cervical mucus** according to either ovulation time (ovulating) or time of progesterone device removal (non-ovulating) in goats and sheep hormonally induced to synchronized estrus

Species	Rates	Hours before or at ovulation time					
		− 60 h	− 48 h	− 36 h	− 24 h	− 12 h	Ovulation
Goat	Sensitivity (%)	0	0	0	0	0	88
	Specificity (%)	100	100	100	75	75	0
		From − 60 h to ovulation or from device removal to 60 h after					
	PPV (%)	64					
	NPV (%)	98					
	ACC (%)	91					
Sheep	Sensitivity (%)	0	0	0	0	0	36
	Specificity (%)	100	100	100	100	91	0
		From − 60 h to ovulation					
	PPV (%)	80					
	NPV (%)	89					
	ACC (%)	88					

* Positive predictive value (PPV), negative predictive value (NPV), accuracy (ACC)

** Cervical mucus type 4 and 5 were used to predict ovulation in goats and mucus type 4 in sheep

and the specificity and the sensitivity were 73% at the ovulation time.

The vaginal epithelial cellular predominance is presented in Fig. 3. In goats, the percentage of nuclear superficial cells increased and those were superior than all the other cell types 12 h before and at ovulation time ($P < 0.05$). Its remarkable predominance at ovulation time (89%) and its low coefficient of variation among observations (6%) drive the use of this rate as the minimum accepted to define a goat as ovulating. In sheep, the highest standard deviation throughout all period was for anuclear cells, with high coefficient of variation (23.7%), while the other cell types remained stable. The percentage of anuclear cells (25%) was higher at ovulation time compared to the moments before and this rate was used as the minimum accepted to define a sheep as ovulating.

The possibility of applying the vaginal cytology (nuclear and anuclear cell predominance in goats and in sheep, respectively) as a tool to predict the ovulation time is described in Table 2. In goats, the sensitivity at ovulation was 88%. Overall accuracies were 88% (goat) and 67% (sheep). When considering only an approximate period of estrus (36 h before ovulation), the accuracies were 78.4% (goat) and 61.4% (sheep).

Discussion

The hypotheses of this study were partially proven. In goats, both techniques (vaginal cytology and mucus evaluation) were similarly capable of reaching high accuracy to determine

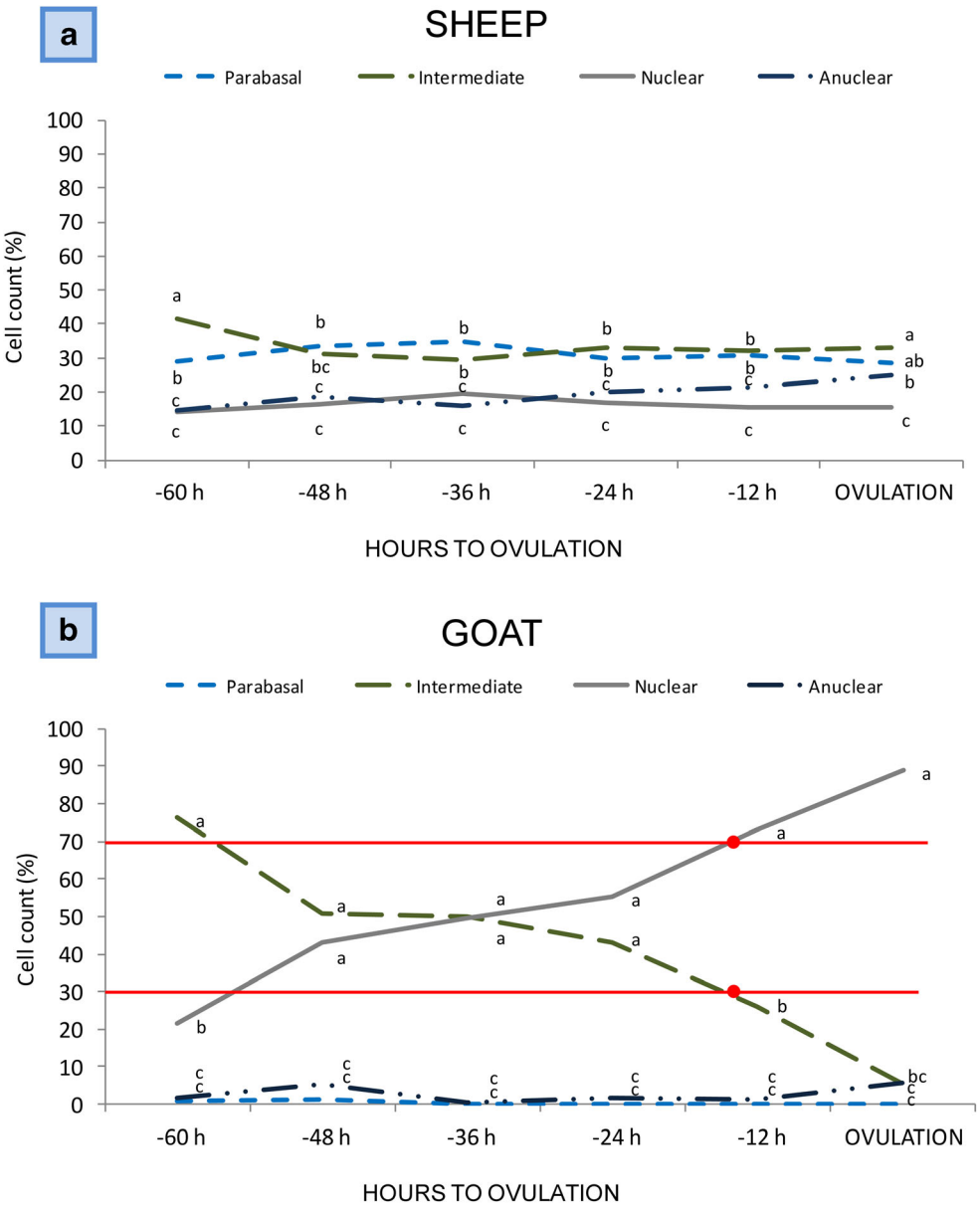

Fig. 3 Vaginal epithelial cellular predominance (%) in hormonally estrus induced sheep (**A**) and goats (**B**) from 60 h before to the ovulation detection by ultrasonography. a,b,c ($p < .05$)

Table 2 Performance parameters* of vaginal cytology** according to according to either ovulation time (ovulating) or time of progesterone device removal (non-ovulating) in goats and sheep hormonally induced to synchronized estrus

Species	Rate	Hours before or at ovulation time					
		− 60 h	− 48 h	− 36 h	− 24 h	− 12 h	Ovulation
Goat	Sensitivity (%)	0	0	0	0	0	88
	Specificity (%)	100	100	88	88	75	0
		From − 60 h to ovulation or from device removal to 60 h after					
	PPV (%)	54					
	NPV (%)	98					
	ACC (%)	88					
Sheep	Sensitivity (%)	0	0	0	0	0	55
	Specificity (%)	91	64	82	45	64	0
		− 60 h to ovulation					
	PPV (%)	26					
	NPV (%)	88					
	ACC (%)	67					

* Positive predictive value (PPV), negative predictive value (NPV), accuracy (ACC) ** The minimum accepted to define the ovulation time was 89% of nuclear cells for goats, and 25% of the anuclear cells for sheep

ovulation time. However, in sheep, the vaginal cytology had low accuracy while the cervical mucus was more valuable to be used as a predictor of ovulation time. Surely the US technique is the golden method to perform this procedure, and besides the ovulation time, it also detects other aspects such as number of ovulations, among others. However, the development of less labor-intensive methods is of great importance. For the first time, it is reasonable to assume that simpler and cheaper associated strategies to determine the ovulation time may be applied in small ruminants, especially in goats.

The association between the cervical mucus evolution and ovulation was previously demonstrated in goats (Fonseca et al. 2017). It is noteworthy that in the current study, at ovulation time, besides the higher prevalence of mucus type 4, there was an increase in the number of nuclear superficial (≥ 70%), while the intermediate cells were in lower number (≤ 30%). This increase in the superficial cells at estrus phase was earlier described (Ola et al. 2006). This cell pattern found in goats suggests that together with cervical mucus, vaginal cytology assessment may accurately detect ovulation time. Considering both criteria, the ideal moment to perform AI in goats is when the cervical mucus is between 3 and 4 and the nuclear superficial cells is approximately 70%.

In sheep, the results were in general less accurate. It was difficult to establish a pattern to follow, even when both techniques were associated. Vaginal cytology had low accuracy while the cervical mucus was slightly better to be used as a predictor. When cervical mucus was assessed in ewes either estrogenized or not, the amount of mucus produced was similar (Rexroad Jr and Barb 1977). This may indicate a low potential in mucus secretion in estrus-ewes and lower stratification of vaginal epithelium. This lower quantity of cervical mucus in ewes, compared to goats for example, may be explained by their great mucus retention in the eccentric cervical rings (Kershaw et al. 2005). At ovulation time, most ewes were presenting mucus type 3 and 4; however, there were also ewes showing type 2. In vaginal cytology, parabasal and intermediate cells remained stable throughout the study. The predominance of intermediate cells (> 80%) was earlier reported in ewes in estrus (Ribeiro et al. 2019). Differently from goats, there was an increase in the anuclear cells, but not in the periovulatory time. These findings highlight the need to expand knowledge about the physiology that involves morpho-functional transformations of the cervix and vaginal epithelium during estrus in sheep. According to the results of the present study, it is possible to encourage the use of both vaginal cytology and mucus evaluation as feasible tools to determine the time of ovulation in goats; however, both strategies are less accurate in sheep.

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Availability of data and material The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

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Declarations

Ethics approval This study was approved by the Ethics Committee on Animal Use of the Veterinary Faculty, Unigranrio (004/2017), and thus, it was conducted in line with all ethical standards required.

Consent for publication All the authors consent to publish the manuscript.

Consent to participate Not applicable.

Conflict of interests The authors declare no competing interests.

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