Short Communication

Saliva Crystallization in Cattle: New Possibility for Early Pregnancy Diagnosis?

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Abstract

Saliva sampling is a non-invasive, simple and low-cost procedure. The aim of this study was to confirm the presence and changes of saliva crystallization in domestic cattle during synchronized oestrous cycle and early pregnancy. We verified saliva crystallization as a method for early pregnancy diagnosis. Eight Holstein cows were included into the research. The samples were collected daily from 16th day before to 34th day after artificial insemination (in total 51 days). We observed the following types of crystallization: none, dotted, branch-like, fir-like, fern-like and combinations of them and an atypical pattern. We confirmed the presence of saliva crystallization in cattle and its changes during oestrus synchronization process, insemination and post-insemination periods. We found significant differences in pregnant and non-pregnant animals between 20th and 29th day after insemination. We concluded that pregnancy diagnosis by saliva crystallization might be possible but the practical application of this method is currently unfeasible.

Keywords: crystalline patterns; arborisation; ferning; non-invasive pregnancy diagnosis; reproductive cycle

INTRODUCTION

Heat and pregnancy detection are essential in successful management of cattle breeding but it is often quite expensive, time consuming and some needs veterinary assistance. Obtaining of saliva is very simple and it can be performed by a breeder. Crystallization, also called ferning or arborisation, was described in vaginal mucus (Noonan et al. 1975), nasal mucus (Peterson 1984), saliva (Pardo-Carmona et al. 2010), tears (Golding and Brennan 1989), milk or colostrum (Zondek and Rozin 1954). The typical fern crystallization is distinctive near the peak of follicular activity and around ovulation time when oestrogens predominate. However, progesterone suppresses the crystallization (MacDonald 1969, Linford 1974). Saliva crystallization was mainly studied in women (Kullander and Sonesson 1965; Berardono et al. 1993) and we noticed only two studies focused on saliva crystallization in animals - in beagle bitches (Pardo-Carmona et al. 2010) and camels (*Camelus bactrianus*) (Haberová 2010).

The aim of presented research was the confirmation of the presence and changes of saliva crystallization in domestic cattle during synchronized oestrous cycle and early pregnancy and verification of differences in crystallization between pregnant and non-pregnant animals.

Eight Holstein cows aged two and three years with the first calving in 2011, housed at the Czech University of Life Sciences Prague (CULS) Farm Estate Lány – Ruda, were included into the research. They were kept under the same conditions of nutrition and management. All tested cows were found to be free of any abnormalities in their reproductive system and were included into hormonal oestrus synchronization program (Table 1). The pregnant animals were detected by livestock specialist from CULS Farm Estate Lány – Ruda using ultrasonography.

Table 1. Schedule of oestrus synchronization protocol and sample collection

Day of treatment	Date	Event
0	9. 8. 2011	3 ml of Supergestran® inj. (NORDIC Phrama, s.r.o.)
1st	10. 8. 2011	beginning of saliva collection
7th	16. 8. 2011	3 ml of Supergestran® inj. (NORDIC Phrama, s.r.o.)
14th	23. 8. 2011	3 ml of Remophan inj. (Bioveta, a.s.)
17th	26. 8. 2011	artificial insemination
51st	29. 9. 2011	end of saliva collection

The samples of saliva were obtained daily from 16th day before artificial insemination (AI) till 34 days after AI (Table 1). Saliva sampling was done using a clean coffee stirrer (Haberová 2010), samples were consequently smeared on glass slide and air dried at room temperature. The samples were microscopically assessed once a week at magnification × 400. Crystals were classified according to a system adapted by Haberová (2010): none, dotted, branch-like, fir-like, fern-like, mixed branch-like and fir-like, mixed branch-like and fern-like, mixed fir-like and fern-like, mixed branch-like, fir-like and fern-like, atypical. Only one general type of crystallization was described in one sample. The statistical analysis was carried out using the program STATISTICA.CZ 10.0 (StatSoft, Inc.) by Pearson´s χ^2 test. We evaluated 408 samples of saliva for crystallization in total. The presence of crystallization in all tested animals and changes in types of crystallization during various reproductive stages were

confirmed ($p < 0.00001$). Typical types of crystallization noticed in cattle saliva are demonstrated in Fig. 1.

These results corresponded to the study of Haberová

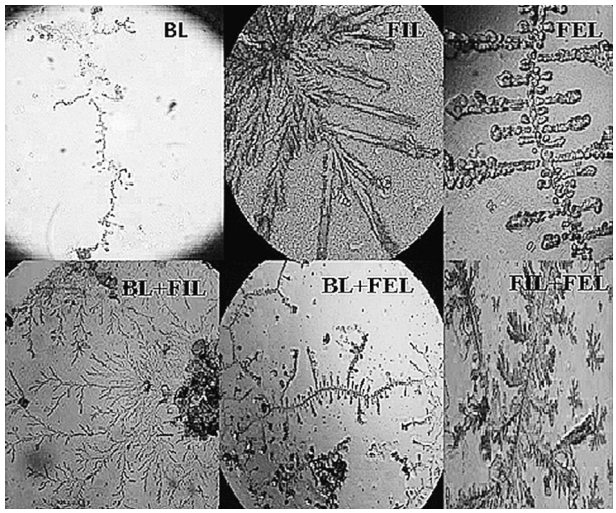

Fig. 1. Typical types of crystallization noticed in saliva of cattle (BL = branch-like, FIL = fir-like, FEL = fern-like). Magnification × 400.

(2010) where all types of crystallization were observed in all tested camels. The majority of crystals based from one point and created irregular "stars". We noticed some similarity in patterns of saliva crystallization with bovine cervical mucus (Noonan et al. 1975), saliva in bitches (Pardo-Carmona et al. 2010) or human tears (Golding and Brennan 1989). Similar to study of Haberová (2010), branch-like crystallization belonged among the most frequent types with overall incidence of 36.27%. Only two types of crystalline patterns were observed during insemination period (Fig. 2), namely

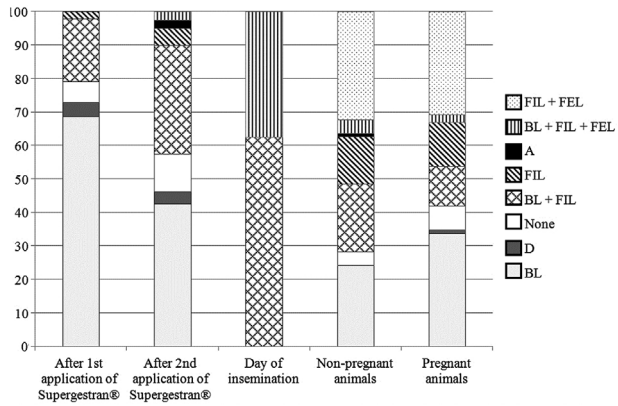

Fig. 2. The distribution (%) of predominating types of crystallization in cattle saliva (n = 404) during different periods (0 = none, D = dotted, BL = branch-like, FIL = fir-like, BL + FIL = mixed branch-like and fir-like, BL + FIL + FEL = mixed branch-like, fir-like and fern-like, A = atypical).

mixed branch-like and fir-like and mixed branch-like, fir-like and fern-like. Similar like in Noonan et al. (1975), crystallization during oestrus was well developed. Fern-like and mixed branch-like and fern-like patterns occurred only after insemination with incidence 1.1% and 0.4%, respectively. Atypical crystallization was first observed in stage after the 2nd application of Supergestran®.

No significant differences ($p > 0.05$) in type of crystallisation were found between pregnant and non-pregnant cows in periods between 1 - 10, 11 - 19 and 30 – 34 days after AI but the type of crystallization was significantly different between 20 and 29 days after AI (p = 0.0410) (Fig. 3). The difference between observed and

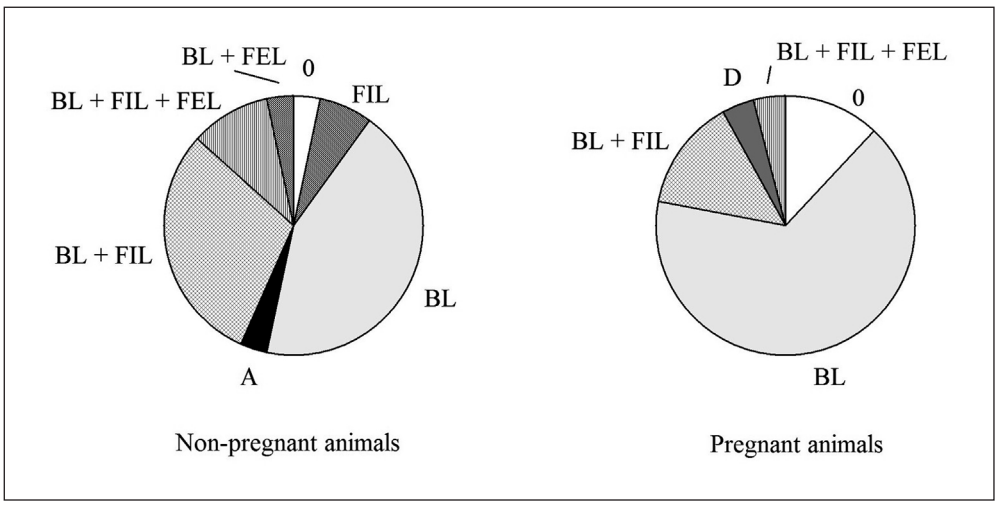

Fig. 3. The differences in distribution (%) of crystallization patterns in pregnant and non-pregnant cows (n = 80) between 20th and 29th day after artificial insemination (0 = none, D = dotted, BL = branch-like, FIL = fir-like, BL + FIL = mixed branch-like and fir-like, BL + FEL = mixed branch-like and fern-like, FIL + FEL = mixed fir-like and fern-like BL + FIL + FEL = mixed branch-like, fir-like and fern-like, A = atypical).

expected frequencies showed the trend that none or branch-like crystallization predominated in pregnant animals whereas fir-like, mixed branch-like and fir-like and mixed branch-like, fir-like and fern-like were presented relatively more often in non-pregnant animals in this period. This finding corresponds with the fact that non-pregnant cows return to oestrus approximately 21 days after AI and the gestation is often confirmed at this time by the determination of progesterone level in blood or milk (Noakes et al. 2001; Hafez and Hafez 2000).

CONCLUSIONS

We confirmed the presence and changes of saliva crystallization in domestic cattle during pregnancy and also in non-pregnant animals. We found significant differences in crystalline patterns in pregnant or non-pregnant animals between 20 and 29 days after AI. Our result indicates that pregnancy diagnosis by saliva crystallization in cattle might be possible; however, the practical application of this method is currently unfeasible. We recommended further and more detailed research focused on this phenomenon.

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