The Veterinary Journal 199 (2014) 471–472

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Short Communication

A new dried milk sampling technique and its application for progesterone detection in cows

Jeanne V. Samsonova *, Alexander P. Osipov, Sergey E. Kondakov

Faculty of Chemistry, M.V. Lomonosov Moscow State University, 119991 Moscow, Russia

ARTICLE INFO

Article history:
Accepted 23 October 2013

Keywords:
Dried samples
ELISA
Pregnancy diagnosis in cows
Progesterone
Whole milk

ABSTRACT

A new method for milk sample collection and storage, based on a dried milk sampling technique, is proposed. The method includes application of a whole milk sample to a porous membrane followed by drying. One hundred whole milk samples (dried and liquid) taken on day 21 post insemination were analysed for progesterone by ELISA and results for both dried and liquid samples were well correlated ($r = 0.911$). Milk progesterone ELISA accuracy for pregnancy diagnosis in cows was 87%.

Early pregnancy diagnosis in cows is vital for profitable dairy husbandry as it helps to improve reproductive efficiency and shorten calving intervals. Any method of pregnancy diagnosis such as rectal palpation, ultrasonography, and measurement of reproductive hormones or pregnancy associated substances has advantages and disadvantages in terms of testing time and accuracy of diagnosis (Purohit, 2010; Lucy et al., 2011). Immunochemical methods based on progesterone determination in milk or serum offer the earliest methods of pregnancy diagnosis in cattle based on the differentiation of pregnant and non-pregnant cows 19–21 days post insemination (Laing and Heap, 1971). At this time point (end of the oestrus cycle) progesterone concentrations in the milk of non-pregnant cows should be low whereas in the pregnant cow it will be high (>7 ng/mL).

Elevated progesterone concentrations 19–21 days after insemination can result not only from a functional corpus luteum of pregnancy but also due to some reproductive disorders (for example, a luteal cyst or persistent corpus luteum), delayed return to oestrus, breeding at the wrong time. So the accuracy of pregnancy diagnosis using a progesterone test 19–21 days after insemination is usually only about 80% (Purohit, 2010). However, as cows cannot be pregnant if they have a low progesterone concentration the method has a high sensitivity for detecting non-pregnant cows (Purohit, 2010). Several milk and serum progesterone enzyme-linked immunosorbent assay (ELISA) tests (quantitative or semi-quantitative) are currently available commercially (Nebel et al., 1989). Milk samples can be analysed either on farm or transported to a laboratory (Posthuma-Trumpie et al., 2009).

In recent years, dried blood spot (DBS) technology has been increasingly used for blood and serum sampling in medical and veterinary practice (Demirev, 2013). This is based on the collection of serum or blood drops on a porous carrier followed by spot drying and transportation to a specialised laboratory for analysis, and the technique, for instance, has been used for large scale neonatal screening for genetic diseases (McDade et al., 2007).

In the present article, we describe the use of a new dried milk sampling technique (DMST) to measure milk progesterone concentration that overcomes the difficulties associated with transportation of whole milk samples to a specialised laboratory. DMST is based on the preparation of dried milk samples on a porous membrane followed by sample analysis in a laboratory after desorption from the carrier.

Whole milk samples from 100 Holstein Friesian cows were taken on day 21 post insemination and analysed by competitive ELISA for progesterone (ELISA-progesterone-milk, Immunoved). Milk samples were collected in clean tubes at the end of afternoon milking and prepared as follows. One portion of each milk sample was frozen at −20 °C. Another portion was used to saturate a 1 × 5 cm strip of membrane (DBST card, Immunoved) and the strip was then dried at room temperature. For the analysis, we used a 10 μL aliquot of a defrosted (liquid) sample and manually punched 0.5 cm (Ø) part of the membrane. Dried samples were analysed by the same ELISA kit with some modifications. Samples were analysed in duplicate against a standard curve by direct ELISA. To confirm pregnancy all cows were checked by rectal palpation between days 65–75 post insemination.

* Corresponding author. Tel.: +7 495 9393407.
E-mail address: jvs@enz.chem.msu.ru (J.V. Samsonova).

1090-0233/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2013.10.031

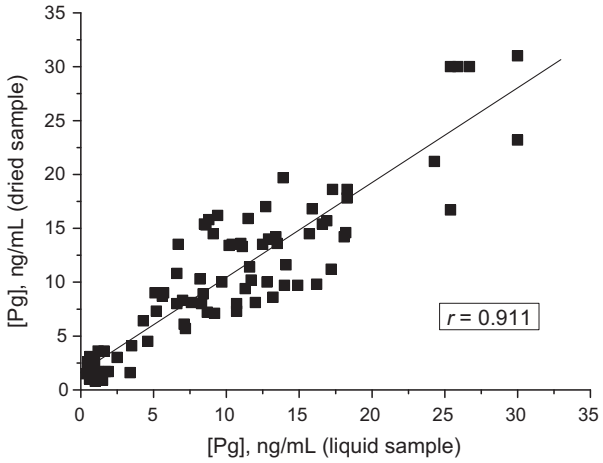

Fig. 1. Determination of progesterone in whole milk by ELISA: correlation between results for liquid and dried samples. Pg, progesterone.

The results of the milk progesterone ELISA recovered from the two types of samples (frozen from fresh or dried) were strongly correlated ($r = 0.911$, Fig. 1) and in full agreement in terms of pregnancy diagnosis. Milk progesterone ELISA accuracy for pregnancy diagnosis in cows vs. rectal palpation was 87%. The ELISA test had a high (100%) sensitivity and negative predictive value irrespective of the type of sample used.

The thermostability of 20 dried milk samples (on a membrane) at ambient and at an elevated temperature (37 °C) was also investigated. Variation of progesterone concentration (between days) did not exceed 10% when samples were stored for 1 week at ambient temperature. Coefficient of variation was within 15% when samples were stored for a week at 37 °C (which is usually considered equal to 180 days storage at ambient temperature or a year storage at 4 °C).

The use of DMST allows all manipulations to be completed on-farm, and allows samples to be easily stored prior to sending to a laboratory by post. The ease of use means that frequent non-invasive monitoring of progesterone becomes significantly more feasible not only for large modern dairy farms but also on smaller farms situated in remote districts.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Acknowledgements

The authors gratefully acknowledge veterinarian Nikolay V. Kireev and artificial inseminator Tatyana N. Rybina (APK Nikulino, Moscow region) for providing whole milk samples, rectal palpation testing and fruitful discussion of the results.

References

Demirev, P.A., 2013. Dried blood spots: Analysis and applications. Analytical Chemistry 85, 779–789.

Laing, J.A., Heap, R.B., 1971. The concentration of progesterone in the milk of cows during the reproductive cycle. British Veterinary Journal 127, 19–22.

Lucy, M., Green, J., Poock, S., 2011. Pregnancy determination in cattle: A review of available Alternatives. In: Proceedings, Applied Reproductive Strategies in Beef Cattle, Joplin, MO, USA, pp. 367–376.

McDade, T.W., Williams, S., Snodgrass, J.J., 2007. What a drop can do: Dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography 44, 899–925.

Nebel, R.L., Altemose, D.L., Munkittrick, T.W., Sprecher, D.J., McGilliard, M.L., 1989. Comparisons of eight commercial on-farm milk progesterone tests. Theriogenology 31, 753–764.

Posthuma-Trumpie, G.A., van Amerongen, A., Korf, J., van Berkel, W.J.H., 2009. Perspectives for on-site monitoring of progesterone. Trends in Biotechnology 27, 652–660.

Purohit, G., 2010. Methods of Pregnancy Diagnosis in Domestic Animals: The Current Status. http://www.webmedcentral.com/wmcpdf/Article_WMC001305.pdf (accessed 21 October 2013).