

EARLY PREGNANCY ASSESSMENT IN DAIRY CATTLE USING RAPID MILK PROGESTERONE (P4) ASSAY

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Abstract

Background: Milk progesterone-based assays may be a valuable tool for the assessment of reproductive performance in dairy cattle, because the milk progesterone levels are directly interconnected with certain fertility events such as estrus, pregnancy, acyclicity or ovarian disorders. The purpose of this study was to evaluate the usefulness of a rapid progesterone (P4) assay for determining early gestation in dairy cows in two family-type farms.

Material and Method: Milk samples were collected from 9 cows in day 18 post artificial insemination, respectively day 21. A small volume of milk sample was collected from each cow in clean Falcon tubes, after the first jets of milk were discarded. Subsequently, 1 ml of milk sample was centrifuged 10000 rpm for 10 minutes. The milk sample was separated into 3 layers and the serum located at the bottom of the tube was recovered with a pipette. A drop of serum was placed in the cassette and the results were read in maximum 5 minutes.

Results: From the 9 females taken into study, 3 were considered non-pregnant and 6 pregnant according to the results of the P4 rapid assay. The non-pregnancy diagnosis was confirmed by rectal palpation at 60 days, while pregnancy was confirmed for 5 out of the 6 cows examined at 60 days post-IA.

Conclusions: The rapid P4 assay may be performed directly by farmers from either milk or urine and the results are available in less than 5 minutes, the ease and reduced costs together with the ability to determine early gestation and assess the reproductive status are making the rapid milk progesterone assay a useful tool for effective management of dairy herds including, smallholder dairy farms.

Key words: early pregnancy; cattle; progesterone; artificial insemination

INTRODUCTION

Milk progesterone-based assays may be a valuable tool for the assessment of reproductive performance in dairy cattle, because the milk progesterone levels are directly interconnected with certain fertility events such as estrus, pregnancy, acyclicity or ovarian disorders (Figure 1). Accordingly, to minimize fertility-related losses on dairy farms, timely identification of the reproductive performance of a cow is crucial. This involves ideal detection of estrus, accurate diagnosis of pregnancy and prompt recognition of early

embryonic death and ovarian disorders [1]. The concentrations of progesterone are high through gestation, thus testing progesterone concentrations 18-24 days after insemination will show pregnancy if progesterone concentrations present high thresholds. However, confirmation of pregnancy by palpation is needed to identify cases of embryonic mortality and exclude other reproductive disorders.

The purpose of this study was to evaluate the usefulness of a rapid progesterone (P4) assay for determining early gestation in dairy cows in two family-type farms.

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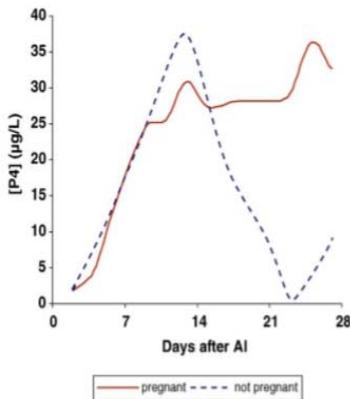


Fig. 1 Progesterone profile (P4) in cow's milk after artificial insemination (AI) according to Pierre Rioux and Denis Rajotte (2004) [7]

MATERIAL AND METHODS

Milk samples were collected from 9 cows within 21 days post artificial insemination. A small volume of milk sample (5 ml) was collected from each cow in clean test tubes, after the first jets of milk were discarded and the samples were left aside for 30 minutes to settle, at room temperature. A small aliquot of milk was taken from the bottom layer of the test tube using the plastic pipette from the kit and 3-5 drops of milk were placed on the test cassette, in the indicated spot (S). The results were read within 5 minutes as described in figure 2 (Figure 3).

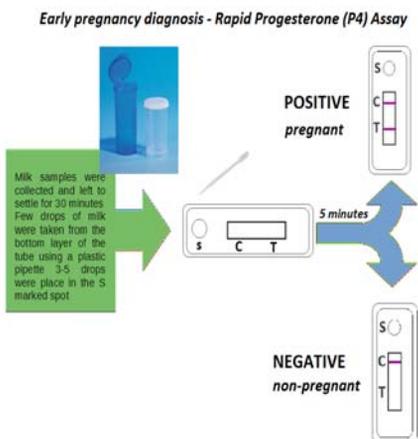


Fig. 2 Graphic presentation of the Rapid P4 Assay for early pregnancy diagnosis; S-sample spot; C-control line; T-test line; The presence of both C and T line indicates a positive result. The presence of only C line indicates a negative result

RESULTS AND DISCUSSIONS

From the 9 females taken into study, 3 were considered non-pregnant since the rapid assay indicated a single line (Control), corresponding to a Progesterone concentration below 2 ng/ml, which is the specificity of the pregnancy test. The 3 females returned to oestrus at in day 22 and 23.

In the case of the other 6 females, the P4 assay indicated a positive result so the tests were repeated at 24 days post insemination when the same positive results were observed. The pregnancy diagnosis was confirmed by rectal palpation, for 5 of the females. Overall, the pregnancy rate determined by the P4 assay and the rectal palpation was 66.66%, respectively 55.55%.

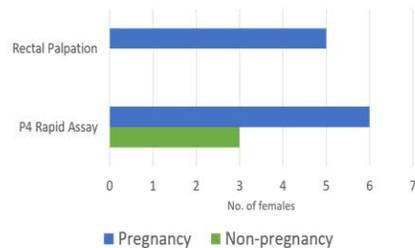


Fig. 3 Pregnancy diagnosis assessed by P4 rapid assay and rectal palpation

In the livestock industry there is a constant pressure for improving performance and ensuring the food demand for animal products, which is expected to increase with 59%-98% by 2050. In this context, the management of reproductive performance is crucial. Various techniques are available for assessing pregnancy status, including return to oestrus, rectal palpation of the reproductive tract and ultrasonography [3], [10].

In practice, return to oestrus method is fraught with issues related to oestrous observation, thereby presently, the most used method for pregnancy detection in cows is ultrasound scanning of the reproductive tract that enables the identification of early embryos and fetal fluids. Although this method is used routinely for assessing the reproductive status in dairy cattle, the investigation is in general performed from day 28 of pregnancy onward. Since the normal oestrous cycle of dairy cattle is 18 to

24 days, rebreeding at the optimal time (i.e., 18 to 24 days post initial AI) for non-pregnant cows is not possible, hence the economic losses associated with this method.

Early identification of pregnant and non-pregnant cows post breeding improves reproductive efficiency and pregnancy rate in cattle by decreasing the interval between services [5]. The rapid progesterone assay allows dairy farmers to detect non-pregnant cows within 21 days after insemination, conveniently before the first heat after artificial insemination, thereby allowing them to reinseminate the females at the first opportunity [3].

For many years, researchers have been testing milk samples to monitor the progesterone concentrations in cattle. The assay used was a very accurate radioimmunoassay, needing special equipment, specific reagents and qualified technicians to perform the analysis. Radioimmunoassay for assessment of progesterone in milk has been commercially used in Europe since 1973, with as many as 100,000 cows being tested in 1980. Nevertheless, the collected samples were submitted to a centralized laboratory for analysis, thus being both time and money-consuming [9].

Studies regarding the bovine estrous cycle have showed that the concentration of milk or serum progesterone may reach a peak value 13-14 days after the estrus and continue to rise afterwards if the female is pregnant [Parkinson et al., 1994]. Low progesterone concentrations at 18 to 24 days after IA may accurately predict non-pregnancy, thereby These elevated concentrations of progesterone in serum or milk between days 18 and 24 after insemination are the foundation for pregnancy diagnosis in cattle [Sasser et al., 1987].

Pregnancy loss is a significant cause of infertility in dairy cows, a source of financial loss and a major challenge for modern livestock systems. According to studies, 10-16% of the confirmed pregnancies at 28 days, results in embryonic death by day 56 post-AI, usually the loss of the conceptus occurring during the first 42 days of pregnancy [11], [12].

Advances in early embryonic or pregnancy detection may lead to strategies to overcome early pregnancy losses therefore

the development of specific, sensitive, accurate, rapid, low-cost, and easy-to-use diagnostic tools is still highly demand.

Nano diagnostics, defined as the use of nanotechnology in therapeutic applications, has been widely researched and employed as an innovative approach to satisfy the requirements of high sensitive clinical diagnosis and early detection. Nanomaterials are now used for the fabrication of rapid assay that may detect various constituents, including progesterone [13]. The rapid assays are basically a sandwich-style ELISA, their format being similar to conventional (plasma or serum) and milk tests but some steps are combined, therefore no special reagents, centrifugation or incubation are needed to perform the analysis.

In the present preliminary study, the rapid progesterone assay enabled us to accurately detect 3 non-pregnant cows. Other 6 cows were diagnosed as pregnant based on the assay results in day 21. The assays were repeated in day 35 and the positive results were reconfirmed. However, the rectal palpation exam confirmed pregnancy for 5 of the cows examined. One possible cause may be embryo mortality, various reports showing that in Holstein – Friesian (HF) dairy cows, early pregnancy losses is estimated to range between 35–45% while late pregnancy losses is around 10%. An association between inadequate maternal progesterone during early pregnancy and early embryo loss was highlighted by several studies [4], [6], [8].

Progesterone (P4) is regarded as the gold standard for the assessment of reproductive performance in cows [2], additionally offering herdsmen a valuable non-invasive way of determining the gestational state of dairy cows.

Yet, there are some limitations using this technique. Although the test is accurate in detecting non-pregnant females, several studies have shown it to be around 75 percent accurate in determining if the cow is definitely pregnant, since elevated values for progesterone 19-24 days post IA may be caused by several conditions other than pregnancy such as uterine infection, some cystic ovarian conditions. Pregnancy

diagnosis should also be confirmed by other methods (i.g. ultrasound or rectal palpation).

CONCLUSIONS

The rapid P4 assay may be performed directly by farmers from either milk or urine and the results are available in less than 5 minutes, the ease and reduced costs together with the ability to monitor ovarian function, making the milk progesterone test a useful tool for effective management of dairy herds including, smallholder dairy farms.

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