A SENSOR BASED AUTOMATIC OVULATION PREDICTION SYSTEM FOR DAIRY COWS

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Abstract
Sensor scientists have been successful in developing detectors for tiny concentrations of rare compounds, but the work is rarely applied in practice. Any but the most trivial application of sensors requires a specification that should include a sampling system, a sensor, a calibration system and a model of how the information is to be used to control the process of interest. The specification of the sensor system should ask the following questions. How will the material to be analysed be sampled? What decision can be made with the information available from a proposed sensor? This project provides a model of a systems approach to the implementation of automatic ovulation prediction in dairy cows.

A healthy well managed dairy cow should calve every year to make the best use of forage. As most cows are inseminated artificially it is of vital importance that cows are regularly monitored for signs of oestrus. The pressure on dairymen to manage more cows often leads to less time being available for observation of cows to detect oestrus. This, together with breeding and feeding for increased yields, has led to a reduction in reproductive performance. In the UK the typical dairy farmer could save £12,800 per year if ovulation could be predicted accurately.

Research over a number of years has shown that regular analysis of milk samples with tests based on enzyme linked immunoassay (ELISA) can map the ovulation cycle. However, these tests require the farmer to implement a manually operated sampling and analysis procedure and the technique has not been widely taken up. The best potential method of achieving 98% specificity of prediction of ovulation is to adapt biosensor techniques to emulate the ELISA tests automatically in the milking system. An automated ovulation prediction system for dairy cows is specified. The system integrates a biosensor with automatic milk sampling and a herd management database. The biosensor is a screen printed carbon electrode system capable of measuring concentrations of progesterone in milk in the range 0.3-2.5 ng/ml. The system is operational in the laboratory is described here and will be working on a test farm in the near future to automatically predict the ovulation of dairy cows routinely.

1 - Introduction

Fertility in dairy cows is a critical component in cost effective dairying. It has been shown over a number of years that optimum financial performance is achieved when cows calve every year\(^1\). The principal method of detecting oestrus is by observation of perturbed behaviour combined with regular recording of fertility events. However as the level of automation at milking has risen (culminating in the introduction of robotic milking in the 1990s) the time and labour available for
observation has diminished. The typical farm in the UK has 100 cows with a calving index of 395 days. Each day over 365 is regarded as costing € 4.8 thus the average farmer could save € 12800 per year by improved oestrus detection. A review in 1997\(^2\) specified a system to improve fertility management in dairy cows with the following features. It should operate automatically with no routine human intervention required. It should be predictive to give the farmer notice that a cow needed insemination. It should be non-invasive – ie it should not require implants or injections. It should detect all events of interest (Sensitivity = 100 %) but not detect events of no interest (Specificity = 95%). It should be aimed at ovulation rather than oestrus. The review concluded that the most appropriate way to meet the specification was to develop a system with sensors to measure progesterone in the milking system combined with a data storage and interpretation system.

The hormonal concentrations found in milk and their relationship to the endocrine system of the cow were reviewed by Pope and Swinburne (1980)\(^3\). Progesterone is a hormone found in whole milk in concentrations from 0 to 50 ng/ml. The concentrations are related to the fat concentration in the milk\(^4\) but as the fat concentration changes little from day to day the change in concentration over time can be used to map the ovulation cycle (Figure 1). Although there are major variations both within and between cows in the concentrations of hormones secreted and the timing of the various events for the majority of cows the features of the ovulation cycle are now well known and described in textbooks\(^5\). In the immediate period post partum concentration of progesterone is low but begins to rise after about 15 days. After the first ovulation, a pattern emerges with a period of 21 days. The ovulation cycle is characterised by a slow rise of progesterone concentration to approximately 20 ng/ml, a plateau of concentration is then reached. At 15-17 days post ovulation the concentration drops to below 5 ng/ml. A few hours after this drop oestrus occurs and 24-48 hours after the drop the cow is ovulating and in an optimum condition for insemination. When the cow becomes pregnant the progesterone concentration rises to a plateau and remains at that level. The concentration of progesterone can thus be used not only to characterise the ovulation cycle but also to detect pregnancy and to diagnose ovarian failure.

ELISA tests suitable for on farm use have been available for a number of years (Ridgeway Science Ltd, Alvington, Gloucs, GL15 6AH, UK) and a considerable number of farmers use them successfully both to confirm oestrus and to detect pregnancy. However, the use of a test kit requires time and skills that are not in abundance on farms and it has been apparent for a while that if a sensor for progesterone in milk could be developed then on-line ovulation prediction would become possible.
Koelsch (1994)\textsuperscript{6} demonstrated a quartz crystal microbalance to detect progesterone in solution. However, the endogenous levels of progesterone were below those detectable with this system. As was pointed out by Claycomb et al, (1995)\textsuperscript{7} the molecular weight of progesterone is too low to significantly perturb the mass of the crystal. Delwiche et al\textsuperscript{8} have also reported methods of detecting progesterone, most recently with an optically read nitrocellulose membrane system\textsuperscript{9}.

An alternative approach was taken by Hart et al (1997)\textsuperscript{10,11} who developed a screen printed carbon electrode surface suitable for binding antibodies. This is used to electrochemically read the results of a competition assay with labelled progesterone mixed in a fixed ratio with milk, followed by incubation, washing and application of an enzyme substrate (Figure 2). This approach has the benefit that the final sensor can be fabricated by printing techniques to deposit layers of materials onto a PVC base. The potential low cost of the disposable sensor, the ease of further development and the simplicity of transduction are important factors in the ability to transfer this technology. Although an ovulation prediction system could use a number of different immunosensor techniques the screen printed carbon electrode sensor is the one that we have chosen for the development of our prototype.

Although we plan to map the ovarian cycle and predict ovulation 24 hours in advance of its occurrence it would be preferable, particularly for diagnostic analyses, to have the results of analysis of the cow’s endocrine status before she leaves the milking stall. A cow usually occupies a milking stall for about 10 minutes. After she enters the milking stall the milker cleans her and attaches teat cups to begin the milking process. In principle a milk sample could be taken immediately and the remaining 8 minutes used to perform the assay. However, following Pope et al (1976)\textsuperscript{4} we conducted tests to determine the change in progesterone concentration during milking. Our preliminary conclusions are that progesterone measurements taken before 120s after the start of milking tend to underestimate the concentration of progesterone found in the whole milk sample, particularly where the endogenous concentration is above 10 ng/ml. Thus for real time analysis we have to reduce our assay time below 6 minutes. If this cannot be achieved then a system of backing up the samples and relating the relevant data to individual cows will be necessary.

The correlation between fat concentration and progesterone\textsuperscript{4} must also be considered. As milk at an afternoon milking of a cow differs from that drawn from the same cow at morning milking\textsuperscript{12} it will be preferable to take samples from the
cow at the same milking time on each sampling day. The variability of fat concentrations between cows may mean that individual thresholds for the concentrations of progesterone may need to be estimated and stored for each cow. Thus the herd management database may need a training algorithm that would analyse samples taken in the period up to 60 days post-partum possibly in conjunction with an analysis of a sample to estimate milk fat from the individual cow. It might also be necessary to include the time since last milking as a factor in calculating the expected concentration of progesterone. It would seem appropriate to sample cows at afternoon milking so that the information can be used to specify cows for insemination or veterinary inspection the following morning.

Although the system will have to operate automatically during milking some servicing is likely to be necessary. Provision will have to be made for sensors to be loaded possibly in a cassette or strip. The system will need some form of regular calibration. This could be achieved automatically by having a reservoir of a analogue of milk with a known concentration of progesterone. The calibration liquid could be treated as a sample at regular intervals and a warning issued if it failed to be accurately measured. Ensuring the repeatability of the sensors would be a major responsibility of the manufacturer’s quality control procedures.

The specification of an automated ovulation system can be stated as a system that will measure concentrations of progesterone in whole fresh milk in the range 1 –30 ng/ml in under 6 minutes. The precision should be as good as a farmer’s ELISA test against which it can be compared. The system should be automatic and linked to a herd management database to give simple outputs to the farmer on demand and at a regular time each day.

**Principles of operation of the automated ovulation prediction system**

When a cow is being milked her identity is known by reading her transponder (or by keying in her ID in a manual milking system). Her individual record is stored on a computerised herd database and will be retrieved and used to determine whether a milk sample is to be taken using a protocol similar to that shown in Figure 1. The protocol will, if necessary, be optimised for automated sampling to include data about fat concentrations, individual cow factors and others as appear necessary and incorporated into a software module that can run within the herd management computer. If a sample is to be taken then a new sensor will be placed automatically into the sampling chamber. A milk sample is drawn from the milk flowing from the cow at some time during milking but probably at about 120s from the start.
With the current sensor the milk sample is mixed in a known ratio (currently 3:5) with an enzyme conjugated progesterone standard solution. The mixture is injected into the chamber. Here it interacts with the immobilised antibodies to progesterone on the screen printed sample electrode surface. After a set time (still to be optimised) the mixture of milk and standard containing unbound progesterone are rinsed from the electrode by pumping buffer through the chamber, leaving labelled and native progesterone bound to the antibodies. A substrate for the enzyme, alkaline phosphatase, (currently 1.00 mM 1-naphyl phosphate\textsuperscript{13}) is then injected into the chamber. The greater the concentration of native progesterone in the milk sample, the more effectively it will compete for the antibody binding sites and so the less conjugated progesterone is present. The level of enzyme present is determined by applying a small potential (currently 300mV) to the auxiliary electrode, after a set time of incubation. A current flows that is proportional to the amount of naphthol released from the 1-naphyl phosphate by the alkaline phosphatase. This current is inversely proportional to the amount of progesterone present in the milk sample. The signal is recorded and then the process is repeated with a blank sensor without the antibody surface. Currently this base calibration is carried out in series with the assay, in future it will operate in parallel to ensure high speed. The concentration of progesterone is calculated for each sample by subtracting the output from the base sensor when exposed to the sample from that for the full sensor and applying a calibration curve. The concentration of progesterone is then passed electronically to the herd database for analysis and reporting to the farmer.

Alternative methods of measuring concentrations of progesterone will be investigated to minimise the complexity of the system and the time of operation. It may be necessary to reduce precision in order to increase speed. The current cycle time of the sensor is in excess of our target of 6 minutes and there are a number of techniques both physical and chemical that we will be investigating with a view to speeding up the incubation.

Once the progesterone concentration is known the stage of ovarian cycle will be determined. Once the drop in progesterone concentration has been determined it may be necessary to take further samples to confirm ovulation. This is particularly important where the concentration is within the range 4-8 ng/ml or where the concentration does not fit within the predicted pattern. Samples could be assayed with a different sensor for other markers of ovulation such as luteinising hormone.
Discussion

This system is currently under development in the laboratory. Our efforts are currently concentrated on optimising the fabrication process of the sensors to ensure the repeatability of the measures. We currently make sensors in batches of 25 and test them daily. Once fabrication is standardised we expect it to become a fully automated process. We shall be testing the system in field conditions later this year.

The frequency of use of the system can be predicted. The most intensive use of the system would come in a herd all calving within two ovarian cycles (six weeks) of each other. If 50 cows in this herd were milked through a robotic stall and each needed 20 assays to characterise the cycle then approximately 1000 sensors would have to be deployed in 42 days. Thus the system would have a mean requirement of 24 sensors per day. It would seem logical therefore to design a sensor packaging system that could cope with numbers well in excess of this. One might propose a pack of 50 sensors. At the other extreme a herd with all the year round calving with a multiple points where each milking machine was used only 10 times per day then 200 sensors would be deployed in 365 days. The expected life of the unused sensor then becomes a major issue in the design and fabrication process.

Fertility monitoring is a major problem for livestock farmers who use artificial insemination. There are over 100 million cattle managed in the developed world in this way. The potential sales of sensors could be in millions per year even if only a small proportion of farmers take up the technology. The cost of the sensors will be very sensitive to the numbers sold. However, the analytical system will also be capable of being extended to disease monitoring. Samples of milk could be analysed routinely for analytes secreted in milk such as markers of mastitis infections or antigens of diseases.

This is the first routine application of biosensors to process monitoring in agriculture and may well open up major opportunities for new research. However, the development of the sensor has to be conducted in the light of a thorough understanding of the total system.

Acknowledgements
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References

1 James, A.D., Esslemont, R.J., 1979, The economics of calving intervals, Animal Production, 29, 157-162.
Figure 1. Ovulation Cycle of a Dairy Cow

Samples to find first cycle

Samples to map ovulation

Samples to predict ovulation and detect pregnancy

Pregnant cows give high signal

Cow Inseminated

Time to first ovulation varies but cycles 21 days

Progestosterone in milk ng/ml

Days post partum
Figure 2. Schematic of the operation of a competition assay to measure unlabelled progesterone in milk in a 5 step system using a screen printed carbon electrode immunosensor.
Figure 3 A schematic of the ovulation detection system. The milk meter will probably be actuated at the same time as the milk flow override switch for automatic cluster removal.