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Monitoring reproduction using immunological techniques

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ABSTRACT

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Immunological techniques have been developed to monitor reproduction under practical conditions. Of these, techniques for confirmation of oestrus and diagnosis of pregnancy are of major importance. The milk progesterone test for dairy cattle and the oestrone sulphate test for pigs are considered and a recently developed novel cow-side milk progesterone test is described. Owing to the rapid development of immunosensors for 'real-time' detection of an analyte, the application of immunosensors for on-farm monitoring of reproduction seems possible within a few years.

INTRODUCTION

Antibodies were discovered in 1890, but it took almost 70 years before the first immunoassays with radioactive and non-isotopic tracers were developed. At first these immunoassays were used mainly in biomedical research to measure polypeptide hormones in plasma and other biological fluids. As the realisation grew that immunoassays could be applied for the detection of a variety of other substances too, the application of immunoassays showed a rapid growth. In veterinary and animal science, assays were initially developed for research purposes. With time the possibilities of monitoring reproduction under practical conditions were recognised and utilised.

IMMUNOLOGICALLY MONITORABLE REPRODUCTIVE TRAITS

From a practical point of view, immunological techniques for confirmation of oestrus and diagnosis of pregnancy are of major importance. The specific

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absence or presence of an immunogenic substance in association with the reproductive trait of interest allows for the development of an immunological technique to monitor the trait in question. The majority of currently used immunological techniques to confirm oestrus or to check for pregnancy are based on the cyclic changes in progesterone during the oestrous cycle and the continuously increased progesterone levels during pregnancy. A major drawback of progesterone-based pregnancy diagnosis is the fact that the diagnostic test can only be applied at times when a non-pregnant animal is expected to have a low progesterone level. This is one of the important reasons for the interest in pregnancy-specific antigens (e.g. Sasser et al., 1986; Zoli et al., 1991). However, until now no practically usable pregnancy test for farm animal species has been based on a pregnancy-specific antigen.

With the increasing use of embryo transfer in farm animals, an immunological technique to monitor transplantation of viable embryos might have a commercial application. In this respect an immunoassay for the early pregnancy factor (EPF) might be of interest. EPF is highly specific for pregnancy and has been demonstrated to appear in maternal blood well within 24 h after transfer of a viable embryo (reviewed by Morton, 1985). The production of polyclonal and monoclonal antibodies against EPF has been reported by Athanasas-Platsis et al. (1989). Apart from practical applications, immunoassays for EPF will also allow valuable scientific research concerning the incidence and timing of embryonic mortality in humans and domestic animals.

MONITORING MILK PROGESTERONE IN DAIRY CATTLE

Objectives of monitoring milk progesterone

The main objectives of monitoring milk progesterone in dairy cattle is to verify whether cows are in oestrus at the time of insemination and to detect cows that do not conceive or do not stay pregnant after insemination. Apart from these objectives, Nebel (1988) also mentioned (a) predicting time of oestrus, (b) differentiating types of ovarian cysts, and (c) evaluating response to endocrine therapy, as potential applications of progesterone testing in a reproductive management program.

From postal services to 'real-time' systems

Laing and Heap (1971) were amongst the first to recognise the practical applications related to the discovery of progesterone in the milk of lactating cows. These potential applications stimulated several groups to develop immunoassays for milk progesterone. Since these assays were still for laboratory use only, postal services for farmers were started. Gradually the tests were

simplified, allowing the development of cow-side tests ('dry chemistry systems').

With the available cow-side kits for milk progesterone, milk samples can be tested on site without the need for expensive equipment or time-consuming postal services. Unfortunately some of these tests are still more suitable for use by a veterinarian than by the farmer. A description of eight cow-side tests has been given by Nebel (1988). Several milk progesterone tests, which were commercially available early in 1991, have been summarised by Smale (1991).

The rapid technical development of immunosensors will allow automated 'real-time' measuring of progesterone on a daily basis in the milking parlour within a few years. More detailed information about immunosensors will be given in the last part of this paper.

A novel bovine milk progesterone test

A cow-side progesterone test should be easy to handle, stable, reliable and should give results within a short period of time. Amongst others, the apoenzyme reactivation immunoassay system (ARIS), a homogeneous competitive binding assay, has the potential to fit into a test which meets the previously mentioned demands (Morris et al., 1981; Tyhach et al., 1981; Sommer et al., 1988). In the Netherlands a milk progesterone test based on ARIS has been developed recently. In this progesterone–ARIS, flavin adenine dinucleotide (FAD) is used as the label. The progesterone–FAD conjugate competes with free progesterone in the sample (e.g. milk) for the limited number of binding sites of the progesterone-specific antibodies. Progesterone–FAD conjugate bound by antibody is unable to reactivate apoglucose oxidase, the inactive enzyme that remains after removal of FAD from glucose oxidase. Conjugate not bound by antibody binds to the apoglucose oxidase and reconstitutes the active enzyme. Reactivated glucose oxidase converts glucose to gluconolacton and hydrogen peroxide. The latter thus generated in the assay can be detected by a peroxidase catalysed chromogenic reaction and is directly proportional to the concentration of free progesterone in the sample.

The prototype that has been developed is a two-step test (20 s preincubation between the progesterone antibodies and the free progesterone in the milk is needed), but requires a minimum of handling. Undiluted whole milk can be used and the test result is available within 3 min. An eye-perceptible colour difference exists between 1 and 10 ng progesterone ml^{-1} milk. All reagents are stable for at least 7 months when kept at 4°C. The test functions well with milk temperature varying between 4 and 40°C and with environmental temperature varying between 4 and 50°C (Van der Meer et al., 1990).

Economic aspects of milk progesterone-based oestrus confirmation and pregnancy diagnosis

To our knowledge the economic benefit of progesterone-based oestrus confirmation has not been quantified. Since the proportion of cows not in or near oestrus when inseminated varies among herds from 0 to 60% (Reimers et al., 1985), a substantial benefit might be expected, especially on the farms with a high error rate for oestrus detection. This benefit should result from reductions in the calving interval and the number of inseminations per conception.

As far as progesterone-based pregnancy diagnosis is concerned, recent cost–benefit evaluations (based on simulation studies) have been reported by Ruiz et al. (1989), Pitcher and Galligan (1990) and Oltenacu et al. (1990). Although the assumptions made in these studies might influence the results, they all come to the conclusion that pregnancy diagnosis based on a milk progesterone test can be profitable. According to Oltenacu et al. (1990) a strategy of on-farm milk progesterone testing on Day 19 after insemination, followed by prostaglandin treatment of non-pregnant cows will be profitable, but only if the efficiency of detection of oestrus among cows diagnosed non-pregnant is increased by more than 20% and if the error rate in pregnancy diagnosis is less than 3%. To achieve the latter, the cows which are non-pregnant on the basis of the on-farm progesterone test should be checked again for pregnancy by other methods.

The market for commercial on-farm progesterone kits seems to be much smaller than had been expected. The world market for 1991 had initially been projected to be over $20 million. Early in 1991 it was estimated to be $1 million only (Smale, 1991), although this has probably been negatively influenced by the format of the present tests.

MONITORING OESTRONE SULPHATE IN THE PIG

Objectives of monitoring oestrone sulphate

The economic loss due to sows that do not conceive or do not stay pregnant after insemination is high, especially if they are not detected as being non-pregnant as soon as possible (Meredith, 1988). The embryonic origin of the precursors of oestrone sulphate (E_1S) in the blood of pregnant sows and the early occurrence of measurable quantities, make it a suitable analyte for early pregnancy diagnosis (Robertson et al., 1978).

Accuracy of E_1S-based pregnancy diagnosis

Changes in plasma E_1S concentrations during the first month of pregnancy in sows have been described by several authors (e.g. Robertson and King,

1974; Robertson et al., 1978; Hattersley et al., 1980; Horne et al., 1983). Work that has been done to investigate pregnancy diagnosis based on plasma E_1S concentrations suggests that the accuracies for detecting pregnant and non-pregnant sows (test sensitivity and test specificity, respectively) can both be high if blood samples are collected between Days 24 and 30 after insemination (for a review see Almond and Dial, 1987). In a large experiment (953 inseminations) we have found a test sensitivity and test specificity of 99.4% and 96.9%, respectively (Van de Wiel et al., 1992). The high test specificity is especially appealing, since this parameter is insufficient for most methods of pregnancy diagnosis.

A sow-side test for oestrogens in faeces?

At present a drawback of E_1S-based on-farm pregnancy diagnosis is the necessity to collect a blood sample. Considering this, it is of interest that the concentrations of total unconjugated oestrogens (Choi et al., 1987) and oestrone (T. van der Lende and E.A. Vos, unpublished observations, 1991) in faeces are consistently higher between Days 24 and 30 in pregnant than in non-pregnant sows. Although at present oestrogens have to be extracted from faeces before testing, work is in progress to develop a test which can be used with diluted faeces. The ultimate goal is to develop a reliable sow-side test.

IMMUNOSENSORS FOR ON-FARM MONITORING OF REPRODUCTION

The high specificity of antibody–antigen reactions can be integrated in affinity biosensors. As stated by Hall (1990), the function of a biosensor is "to recognize the existence of a specific chemical in solution or a mixture, and produce a signal that is related to the concentration of the chemical in the solution". In general, a biosensor is the combination of a selector and a detector. In order to be classified as affinity biosensor, the selector must contain biological material which can selectively bind an analyte. In the case of immunosensors, antibody–antigen reactions are detected in 'real-time' due to physical changes at a transducer. This physical change can be transduced from the biochemical domain to the electrical domain either via an optical, electrochemical or acoustical pathway.

At present the surface plasmon resonance (SPR) immunosensor is the most developed affinity biosensor (Daniels et al., 1988). Antibody–antigen reactions cause a change in the refractive index at a metal–liquid interface, which results in a measurable change in the reflection intensity of a laser beam. Recently Severs et al. (1992) showed that a model immunoassay (ELISA) can be converted to a SPR immunosensor, introducing several advantages such as a fast response, 'real-time' detection, minimal handling and low cost per test

Commercial cow-side progesterone SPR immunosensors will probably be accepted rapidly due to the intrinsic benefits of highly developed immunosensors. However, for each immunosensor application, including on-farm progesterone immunosensors, an intensive research and development effort has to be invested to meet the requirements and specifications. For the detection of low molecular weight molecules such as progesterone, the use of a refractive index label is inevitable to meet the requirements of high sensitivity and specificity. Immunomodified latex particles in a competitive assay format have been used for the enhancement of the signal (Schasfoort, 1990). If the selector can be regenerated, it is expected that the progesterone status of cows will be monitored automatically in the milking parlour within a few years.

Based on the large commercial investments of diagnostic companies in SPR products it is expected that the SPR potential will reach a $15 billion market in 1995 (including clinical diagnostics). The near future will show how large the impact of (SPR) immunosensor technology for diagnostics and monitoring will be.

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