Pregnancy diagnosis in dairy goats and cows using progesterone assay kits

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SUMMARY: Early pregnancy diagnosis in dairy goats and cows was studied using enzyme immunoassays (EIA) to measure progesterone concentratIons in whole milk samples col1ected approximately 3 weeks after mating. Two qualitative on-farm assay kits and 2 quantitative assay kits, al1 designed for use In the dairy cow, were tested for their accuracy with goats milk samples. Accuracy of dIagnosis of goat pregnancy ranged from 83 to 88%, and of non-pregnancy from 80 to 100%. Pregnancy dIagnosIs with samples of cows milk using 2 quantitative kIts gave accuracies of 66 to 68% for pregnancy, and 90 to 910/0 for non-pregnancy. Possible causes of error in the early diagnosIs of pregnancy with milk samples are discussed.
Aust Vet J 68: 14-16

Introduction

The early diagnosis of pregnancy is an important aspect of reproductive management of a dairy herd. It can affect profit through factors 'such as the amount of milk produced per day of herd life. decisions on culling and feeding regimes, and the identification of fertility problems. With dairy goats in particular, early diagnosis is important because of their seasonal breeding pattern (Baxendel1 1986).

Since Laing and Heap (1971) first described the use of milk progesterone measurements as an early indicator of the reproductive status of the lactating dairy cow, there has been considerable research on the development of sensitive and reliable assays for progesterone in milk. Early studies concentrated on radioimmunoassay (RIA) techniques (Heap *et 01 1976;* Pennington *et 011976;* Holdsworth *et 011979),* but these require use of a radioactive label and a central testing laboratory equipped with specialised instrumentation. More recently, enzyme immunoassays (EIA) have been preferred (Sauer *et 011981;* Munro and Stabenfeldt 1984; Marcus and Hackett 1986; Nebel *et 01(987)* as they offer advantages of safety, reagent stability and on-farm application.

The accuracy of milk ,progesterone EIA for early pregnancy diagnosis (19 to 24 d after mating) in dairy cows has been reported at 70 to 950]'0, and for non-pregnancy at 80 to 1000]'0 (Cleere *et 011985;* Wimpy *et 011986;* Nebel *et 011987;* Worsfold *et 0/1987).* In dairy goats, trials using RIA to measure milk progesterone in samples 20 to 26 d after mating gave accuracies of 71 to 980]'0 for pregnancy diagnosis, and 90 to 1000]'0 for non-pregnancy diagnosis (Holdsworth and Davies 1979; Pennington *et 011982;* Thibier *et 01(982).* No study using milk progesterone EIA to diagnose pregnancy in dairy goats has been reported.

Similarities in oestrus cycle length between the dairy goat and dairy cow (21 to 22 d), and in milk progesterone concentration make it possible to use EIA kits designed for bovine applications with dairy goat samples. This study was designed to test the accuracy of bovine milk progesterone kits in determining the reproductive status of both goats and cows 3 weeks after mating.

Materials and Methods

Milk Samples

Samples of goats milk were collected from 3 commercial dairy herds in south-east Queensland on the day of mating (day 0, oestrus sample), and 21 to 24 d after mating (pregnancy sample). Samples of cows milk from 4 commercial dairies and the Department of Primary Industries' research herd at the Animal Genetics Centre. Harrisville. were collected 22 d after mating (pregnancy sample). A composite whole milk sample (10 to 20 ml) was collected from each animal, preserved with potassium dichromate and stored at 5°C until analysis.

Progesterone EIA Kit Assays

The following commercial kits were used for progesterone assays: Calfcheck Reprostrip., Noctech Progesterone EIA., Ovucheck Cowsidet, Ovucheck Milk Progesterone EIAt and Enzygnost\!) Milk Progesterone Vett. Kit components were warmed to room temperature prior to use. and the assays performed according to the manufacturer's instructions. For qualitative assays (Calfcheck Reprostrip, Ovucheck Cowside), results were interpreted visually by comparison with controls, while for quantitative assays (Noctech Progesterone El A, Ovucheck Milk Progesterone EIA and Enzygnost Milk Progesterone Vet), absorbances of test and standard samples were measured on a microtitre plate reader§.

Classification of oestrus and pregnancy samples was done according to the kit instructions, with the following 2 exceptions: I) with Noctech EIA, a cut-off of 5 ng/ml progesterone was used.

For oestrus samples with progesterone < 5 ng/ml, oestrus was confirmed; for pregnancy diagnoses, samples with progesterone < 5 ng/ml were classified non-pregnant, and those) 5 ng/ml were classified pregnant.

2) with Enzygnost Milk Progesterone Vet, pregnancy samples with progesterone concentrations between 5 and 10 ng/ml, considered borderline by the manufacturer, were classified non-pregnant.

Trials

In Trial I, samples of goats milk were assayed for their progesterone concentration using a quantitative assay kit (Noctech Progesterone EIA). Trial 2 tested 2 qualitative EIA kits (Calfcheck Reprostrip and Ovucheck Cowside) and a quantitative EIA (Ovucheck EIA) with goats milk samples. In this trial, the pregnancy sample was included in the results only if oestrus was confirmed in 2 or more assays.

Samples of cows milk were tested in Trial 3, where 2 quantitative progesterone assays (Ovucheck EIA and Enzygnost Vet) were used.

Pregnancy Diagnosis

The reproductive status of goats was recorded by the farmer, and involved noting a return to oestrus, live birth, or in some cases, false pregnancy or abortion. Goats were assumed to be not pregnant if they were observed by the owner to return to oestrus after service at the oestrus confirmed by the milk progesterone assay. Goats were assumed to be pregnant to the service at the oestrus confirmed by the milk progesterone assay if they gave birth to a full term kid 148 to 156 d after this service. Cows were tested for pregnancy by rectal palpation at 60 d after mating. For the purposes of analysis, on-farm diagnoses were assumed to be 1000]'0 accurate.

.' Results

Pregnancy Diagnosis in Dairy Goals

Trial J – Thble I shows the results of Trial I, in which the accuracy of pregnancy diagnosis for a quantitative EIA (Noctech) was determined for goats milk samples collected 21 to 24 d after

• Noctech Ltd, Dublin, Ireland
t Cambridge Life Sciences, Cambridge, England t Hoechst Animal Health, Milton Keynes, England § Titertek Muhiskan MC, Flow Laboratories, Sydney

mating. All does were sampled on the day of breeding, and the EIA confirmed that all were in true oestrus, with a mean progesteronelevel of 0.44 ng/ml (range 0 to 2.8 ng/ml). The mean progesterone concentration of pregnancy samples classified non-pregnant was 0.77 ng/ml (range 0 to 4.0 ng/ml), and of samples classified pregnant 20.7 ng/ml (range 6.5 to 35.0 ng/ml).

Overall accuracy of the EIA was *96.4"70* (27 of 28), with non-pregnant interpretations 1000(0 accurate. The sample giving a false positive result was recorded as a false pregnancy, or "cloudburst" (a pseudopregnancy terminated by the sudden evacuation of a large volume of fluid from the uterus). Based on the results of the trial and the progesterone levels of samples classified pregnant and non-pregnant, 5 ng/ml appears a suitable cut-off for discrimination between samples assayed by this EIA.

Trial 2 -1\vo qualitative, on-farm EIA kits (Calfcheck Reprostrip and Ovucheck Cowside) and one quantitative EIA (Ovucheck EIA) were tested using- goats milk samples in Trial 2. Samples collected on day 0 (oestrus sample) showed that 4 does were not in oestrus at the time of mating, with all 3 kits giving a negative result. The pregnancy samples from these does were rejected as they would give a false positive result. About onethird of the oestrus samples tested with Ovucheck Cowside gave a negative result, yet tested positive with the other 2 kits. The Cowside results proved incorrect in many cases, as the does subsequently kidded, and therefore must have been in oestrus
on day O.

The results of the trial (Thble 2) indicate that all 3 kits had good accuracy in predicting pregnancy and non-pregnancy. Overall accuracies for Calf check Reprostrip, Ovucheck Cowside and Ovucheck EIA were 87.9, 86.2 and 85.40/0, respectively. Ovucheck Cowside, while not reliable for predicting oestrus in goats milk samples, had 100"70 accuracy for non-pregnancy diagnosis.

Pregnancy Diagnosis in Dairy Cows

Trial 3 - The accuracy of progesterone assays in diagnosing pregnancy in samples of cows milk 22 d after mating was assessed with 2 quantitative EIA kits, Ovucheck EIA and Enzygnost Vet (Table 3). The mean progesterone' concentrations of samples classified non-pregnant by these tests were 1.0 and 3.3 ng/ml, respectively, (ranges 0 to 4.4 ng/ml and 0.7 to 9.4 ng/ml, respectively). Mean levels for samples classified pregnant were 20.7 and 27.5 ng/ml, respectively, (ranges 5.7 to 32.9 ng/ml and 10.8 to 49.9 ng/ml, respectively).

Kit instructions accompanying Enzygnost Vet suggest that cows giving borderline results (between 5 and 10 ng/ml) be retested the next day. In this trial, 5 borderline results were classified as non-pregnant; all cows were subsequently found to be non-pregnant.

While the overall accuracy for both kits approached *80"70,* an analysis of the results on a farm basis suggests that a higher figure may be achieved (Table 4). Using Enzygnost Vet, all farms had 100"70 accuracy except farm 4 in Table 4 *(64"70).* Ovucheck EIA also gave very high accuracies on all farms except farm 4. The two false negative results for both kits were also from this farm. These results suggest that the lower accuracy of milk progesterone assays on samples from farm 4 arises from a problem peculiar to that farm.

Discussion

The use of progesterone assays as an early indicator of pregnancy in dairy cows is becoming increasingly popular, and with the advent of cowside testing, may become an essential tool for the veterinarian and farmer in making early decisions on culling and rebreeding. On-farm tests are currently designed to give only a qualitative, rather than a quantitative indication of progesterone concentration, and may take only 5 min to complete (Elmore 1986; Nebel 1988). Quantitative tests giving accurate concentrations of progesterone in milk are more suited to the veterinary practice equipped with a microplate reader and micropipettes, and may take up to 1 h.

In the studies reported here, both types of assays were used to determine the accuracy in diagnosing pregnancy in dairy goats and cows. The results show that both kit types, while designed. for bovine applications, are highly accurate and can be used for either species. The unexpectedly low accuracy of assays on cows milk samples from one farm highlights the need for careful attention to details such as sampling and confirmation of oestrus at the time of mating.

TABLE 1
Pregnancy diagnosis in dairy goats using Noctech milk progesterone EIA

Diagnosis '	Number	Numer correctt	% Accuracy
Pregnant	9	8	88.9
Non-pregnant	19	19	100.0
Total	28	27	96.4

, Samples with progesterone concentrations > 5 ng/ml were classified as pregnant, and samples (5 ng/ml as non-pregnant
t Determined from farm records

TABLE 2
Pregnancy diagnosis In dairy goats by milk progesterone EIA

Diagnosis'	Number	Number correctt	% Accuracy
Calfcheck Reprostrip			
pregnant	43	38	88.4
non-pregnant	15	13	86.7
total	58	51	87.9
Ovucheck Cowside			
pregnant	48	40	83.3
non-pregnant	10	10	100.0
total	58	50	86.2
Ovucheck EIA			
pregnant	33	29	87.9
non-pregnant	15	12	80.0
total	48	41	85.4

, Samples classified according to kit instructions t Determined from farm records

TABLE 3
Pregnancy diagnosis in dairy cows by milk progesterone EIA

Diagnosis.	Number	Number correctt	% Accuracy
Ovucheck EIA			
pregnant	29	19	65.5
non-pregnant	22	20	90.9
total	51	39	76.5
Enzygnost Vet			
pregnant	22	15	68.2
non-pregnant	20	18	90.0
total	42	33	78.6

• Ovucheck EIA: progesterone> 5 ng/ml, pregnant and (5 ng/ml, non-pregnant; Enzygnost Vet: progesterone) 10 ng/ml, pregnant and (10 ng/ml, non-pregnant
t Determined from records of rectal palpation

TABLE 4 Analysis of pregnancy diagnosis In dairy cows by milk progesterone EIA on a farm-to-farm basis

Farm	Test Kit: Ovucheck EIA	Enzygnost Vet
1	100.0 (2/2)'	100 (2/2)
2	100.0 (3/3)	100 (3/3)
3	91.7 (11/12)	100 (12/12)
4	60.0 (15/25)	64 (16/25)
5	88.9 (8/9)	NTt

, Percent accuracy - numbers in brackets represent correct diagnoses (pregnant and non-pregnant)/total number tested
t Not tested

As has been noted previously (NebeI1988), accuracy of identifying pregnant animals is usually lower than for identifying non-pregnant animals, since the presence of high milk progesterone levels 21 to 24 d after mating may be due to factors other than pregnancy, for example prolonged luteal phase, luteal cysts or breeding during the luteal phase. In addition, inaccurate sampling may lead to test inaccuracies. Embryonic death post-testing may also lead to differences in results between the test and the final outcome, although the test was correct on the day on which it was performed. False negative results - that is, low progesterone level although the animal is pregnant, are less common, but pregnant cows with exceptionally low progesterone levels (2.1 to 4.2 ng/ml) 21 d after mating have been reported (Marcus and Hackett 1986).

Progesterone assay kits, when used in combination with good farm management practices, can be employed to predict simply, quickly and with accuracy, the pregnancy status of dairy goats and dairy cows. However, a major limitation with these tests when used to diagnose pregnancy is that the animal must be accurately detected on oestrus at the time of mating; if the animal is mated during the luteal phase of the cycle it is likely that she will be in the luteal phase when the pregnancy test sample is collected, and will give a positive assay result. It should also be emphasised that milk progesterone assays are only a management aid and should be used as part of an overall strategy to improve herd reproductive and economic performance.

Acknowledgments

This work was supported by grants from the Australian Special Rural Research Council and the Dairy Goat Society of Australia Research Foundation. The author thanks Mr A Vos and Mr T Schmidt for technical assistance and co-operating farmers for their assistance.

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(Accepted for publication 15 *August 1990)*

BOOK REVIEWS

Necropsy: Procedures and Basic Diagnostic Methods for Practising Veterinarians. AC *Strafuss. Charles* C *Thomas, Springfield, Illinois.* 1988. 244 *pp. $39.75 US.*

This book is wriUen for the veterinary student and the general practitioner, and is prefaced by an eloquent justification of the place of the necropsy in general practice. The necropsy performed in clinical practice remains the single most effective and accessible vehicle for continuing education: teachers of veterinary pathology trot out this truism year after year to undergraduates and anyone else who will listen, but it is nice to see the credo in print

The scope of the book is broad; it covers most domestic species and includes birds. Basic bacteriological techniques extend to cultural and staining characteristics; likewise, some simple toxicological tests are presented. It is perhaps unlikely that, given the current litigious climate in the United States, there are any general practitioners who would not simply send all specimens straight to specialist pathology laboratories. Nevertheless, the inclusion of laboratory procedures will be useful for those independent souls in Ihe Australian backblocks who want to set up some basic laboratory facilities. While on the topic of bacteriology, perhaps more emphasis could have been given to the fact that the best transport medium for pathogens is often the lesion itself (adequately refrigerated, of course). Modern courier services and insulated containers are changing the demands on the submitter of samples, and many bacteriologists feel they can make more meaningful isolations (especially of anaerobes) from the affected tissue itself. Besides, the lesion will always be more interesting than a swab or pipette.

There are sections on the performance of the necropsy and its recording. A lot of attention is given to helping the reader distinguish between real lesions and insignificant *post-mortem* or agonal changes; there will be disagreement among pathologists about some of the statements in this section. For example, the role of fibrinolysis of blood that has coagulated *post-mortem* has apparently been overlooked in the section on interpretation of the heart. Moreover, it is unfortunate that there is no account of the spectrum of changes produced by administration of concentrated euthanasia solutions, either intravenously or into serous cavities. Elsewhere (p144), one might well disagree with pulmonary congestion and oedema being: "Almost never present without an observable lesion to account for its presence . . ."; particularly when this is followed immediately by the assertion that bronchial and tracheal froth is: "Present to some degree in most animals as they die." But, overall, this attention to interpretation addresses the most important stumbling block for the neophyte or occasional pathologist. To recognise normal anatomy and the sequence of autolytic and putrefactive changes may not 'SE!eman intellectual challenge, but a prosector who fails in this recognition is hugely handicapped, and will likely be too frustrated to gain much benefit from the exercise of necropsy. Likewise, the appendix on knifesharpening might not be regarded as appropriate for a modern textbook, but one can only agree with the author's forceful statement: ". . . if the owner (of the animal) can open a carcase with greater expertise, it will be obvious to everyone watching that a pumpkin head has been hired to do the necropsy. The difference between an artist at work and a dolt is a sharp knife". Other appendices include recipes for some stains and a bacteriological identification flow-chart. There are sections on jurisprudence and carcase disposal which, although interesting, are of limited relevance to Australian conditions.

The book is well-bound and presented, but better editing would have led to a tigher and more easily-read text. For example, chapter 4 ("Postmortem Changes versus Antemortem Lesions") could logically have been combined with chapter 12 ("Gross Observations of Diagnostic Significance"). Computer spell-checking has not eliminated some amusing errors, for example, "current jelly clot" on p.53.

Veterinary undergraduate and post-graduate students will find this book useful, as will any veterinary practitioner, particularly those whose failure to regularly perform necropsies has been due to fear of the unknown.

WR Kelly

Ticks and Tick Borne Diseases. RW Sutherst. Argyle Press, Mentone. 1986. 159 *pp.*

This is the proceedings of an international workshop on the ecology of ticks and tick borne diseases held at Nyanga, Zimbabwe. This workshop was sponsored by the Australian Centre for International Agriculture Research.

There are five chapters, which were Introductory Papers, Ecology, Epidemiology, Losses in Production and Management.

The objective of the workshop was to discuss tick-related problems and methods of alleviation in Africa. Many of the papers presented discussed the use, the development and the implication of tick models namely T3HOST, TICK2 and CLIMAX. These models help to desc~ibe the population dynamics of *Rhipicephalus appendiculatus* and *Amblyomma sp,* transmission of tick-borne diseases and tick control methods. Most of the papers presented were only position papers covering aspects of their research.

The workshop evaluated the data available and made recommendations on future research priorities and emphasis.

The African experience as expressed by the papers presented is one of great complexity. Since they are dealing with a multitude of host species, many tick species (1-3 host species), several tick-borne diseases and a tradition;;!1 farming system.

This book is better suited for institutions rather than individuals.

RR McKinnon