Diagnosis of pregnancy in dairy cows based on the progesterone content of milk. Part 1. Discriminating between pregnant and non-pregnant dairy cows on the basis of milk progesterone levels

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A linear discriminant function (LDF) was used to estimate the level of milk progesterone which allowed the best overall classification of dairy cows into pregnant and non-pregnant groups (confirmed by rectal palpation). Progesterone levels were measured in milk samples drawn between 20 and 24 days after insemination. This function, with milk progesterone as the only variable, was tested on a population of 188 pregnant and 103 non-pregnant cows, correctly classifying 98,0% of all cases (98,9% of pregnant cows and 97,1% of non-pregnant cows), when the discriminating level for progesterone was set at 5 ng/ml milk. At the same level of progesterone this function correctly classified 94,4% of all cases belonging to a different sample of dairy cows. This technique appears to allow selection of a milk progesterone level which maximizes accuracy of classification into both pregnant and non-pregnant categories.

'n Lineêre diskriminantanalise (LDA) is gebruik in die bepaling van die melkprogesteroonvlak wat die beste algemene klassifikasie van suiwelkoeie in dragtige en nie-dratige (bevestig deur rektale ondersoek) groepe kon toelaat. Progesteroonvlakke van melkmonsters, wat 20 tot 24 dae na inseminasie verkry is, is bepaal. Hierdie funksie, met melkprogesteroon as die enigste veranderlike, is op 'n populasie van 188 dragtige en 103 nie-dragtige koeie getoets. Teen 'n diskriminante progesteroonwaarde van 5 ng/ml melk het hierdie funksie 98,0% van alle gevalle (98,8% van dragtige en 97,1% van nie-dragtige koeie) in die oorspronklike groep koeie korrek geklassifiseer. By dieselde progesteroonpeil, maar in 'n ander groep koeie, was die klassifikasiekorrektheid 94,9%. Hierdie tegniek vergemaklik die bepaling van die melkprogesteroonvlak waarby die akkuraatheid van klassifikasie van dragtige sowel as nie-dragtige koeie gemaksimeer word.

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Progesterone levels in milk are closely related to the growth and secretory function of the corpus luteum in the normal cycle and in early pregnancy (Darling, Laing & Harkness, 1974). In the non-pregnant cow, as the corpus luteum regresses toward the end of the oestrous cycle, so progesterone levels in milk decline rapidly from day 17 to basal levels (Pope, Majzlik, Ball & Leaver, 1976). However, in the pregnant cow, the corpus luteum persists so that the high levels of milk progesterone normally reached on about day 14 of the oestrous cycle (oestrus = day 0) continue. Milk progesterone levels may thus be used to distinguish between the pregnant and non-pregnant states with the progesterone levels differing most widely on days 20 and 24 after insemination (Heap & Holdsworth, 1981). In order to avoid incorrectly classifying cows that are open as being pregnant at this time, a relatively high minimum level of milk progesterone can be selected to denote the pregnant state. Since cows which are in fact pregnant may then be regarded as barren, the reliability of the classification into the non-pregnant category is reduced. The problem then becomes one of selecting a level of milk progesterone which will maximize the correctness of classification into both pregnant and non-pregnant categories. Discriminant analysis begins with the desire to distinguish between two or more groups (e.g. pregnant and non-pregnant dairy cows). The researcher must select a set of discriminating (predictor) variables that measure characteristics on which the groups are expected to differ (Klecka, 1975). The object is to form a linear combination of the predictor variables with associated weights which will divide observed cases (dairy cows) into groups (pregnant and non-pregnant) that are as statistically different as possible (Hardy & Weed, 1980).

Although linear discriminant analysis assumes that the predictor variables are multivariate normal in each group, non-normality does not necessarily rule out effective data analysis since the technique is robust to such violation (Lachenbruch, 1975; Tabachnick & Fiddell, 1983). Furthermore, Truett, Cornfield & Kannel (1967) suggested that multivariate normality of predictor variables is not necessary, but rather that a less

stringent condition is sufficient, namely that the predicted discriminant function scores, themselves, be univariate normal within each group. This paper describes the application of discriminant analysis in the diagnosis of pregnancy in dairy cows based on the progesterone content of the milk.

Whole milk samples were taken in the evening from cows (in four herds) which had been inseminated 20—24 days previously. The progesterone concentration of each sample was measured using the radioimmunoassay technique devised by Holdsworth, Chaplin & Booth (1979). Cross-reactivity studies with related steroids indicated that the antiserum used was highly specific to progesterone (Butterfield, 1986). The within- and between-assay coefficients of variation were 6,7% and 13,8% respectively. Rectal palpations performed 60 days after insemination were used to establish whether cows were pregnant or not.

The results of 291 pregnancy tests (188 cows diagnosed pregnant and 103 non-pregnant) were used to estimate a linear discriminant function where the level of milk progesterone was the only predictor variable. Discriminant function scores were then predicted for each cow. Plots of the predicted scores exhibited approximate univariate normality within each group. If the score predicted for an individual cow exceeded some critical value the cow was classified pregnant, otherwise the cow was classified non-pregnant. Critical values (cut-off points) were chosen to represent progesterone levels ranging from 4 to 6 ng/ml milk (Hope, 1968). The results of each classification are detailed in Table 1.

Table 1 Classification of known pregnant and non-pregnant cows at different milk progesterone levels using the estimated discriminant function

Progesterone level (ng/ml milk)	Correct classification of cows (%)		Weighted mean	Pearson correlation (Predicted *vs* actual)
	Non-pregnant	Pregnant		
4,0	92,0	99,5	95,8	91,1
4,5	93,2	98,9	96,1	93,2
5,0	97,1	98,9	98,0	96,2
5,5	98,1	97,9	98,0	95,5
6,0	98,1	94,7	96,4	91,3

From Table 1 it is apparent that progesterone cut-off levels of 5,0 and 5,5 ng/ml milk both yielded correct overall classification rates of 98,0% for the original sample. The zero-order correlation between predicted and observed group membership was however higher in the former case. Since the predictive ability of the LDF is likely to be overstated when the same data set is used to estimate both the LDF and its predictive powers (Lachenbruch, 1975), a second sample of 68 cows (from the same herds), diagnosed as pregnant or non-pregnant, was classified using the original LDF and a progesterone cut-off level of 5,0 ng/ml milk. In this partial validation procedure the overall correct classification rate was 94,4% with correct classification rates of 98,3% for non-pregnant cows and 90,4% for pregnant cows. Dobson & Fitzpatrick (1976) and Heap, Holdsworth, Gadsby, Laing & Walters (1976) have obtained similar correct classification rates.

A progesterone content of 5 ng/ml thus provides a useful indicator of pregnancy in local dairy cows. This discriminatory level is similar to that reported for whole milk by Dobson & Fitzpatrick (1976) and Holdsworth *et al.*, (1979). Some workers (Dobson & Fitzpatrick, 1976) have introduced both an upper and lower discriminatory limit, thus allowing some flexibility in diagnoses. Cows having progesterone levels falling within these limits at the time of testing are then classified 'doubtful'.

It was anticipated that the predictive power of the estimated LDF would be lower for pregnant cows relative to non-pregnant cows owing to factors such as embryo mortality, luteal cysts, oestrous cycles of irregular length and incorrect sample collections. Such physiological or managerial factors are easily able to account for the 10% loss in accuracy.

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