# Feasibility study for predicting progesterone levels in cattle plasma by near infrared spectroscopy

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## Introduction

The breeding efficiency is one of the main factors that determine the final profitability of a dairy cattle farm. An adequate reproductive control program should integrate the different aspects of management of the farm, as nutrition, health, etc., stressing on the evaluation of the reproductive indicators. One of the main breeding indexes that allow the evaluation of the reproductive management in farm is the calving interval, that must be about 12.5 months. To achieve this goal, it is necessary to control different aspects, as oestrus behaviour detection, carrying the artificial insemination in the correct time, making an early pregnancy diagnosis or diagnosing reproductive pathologies. Knowing the hormonal levels can be an useful tool for the different aspects to control.

The progesterone is an hormone that can be considered as an objective and accurate sensor of the cow sexual function, and because of that is normally used as one of the parameters to control in order to improve the breeding efficiency.[1] Their release pattern and their high levels in blood during the luteal phase cause that the progesterone be the most used hormone for monitoring the ovarian activity in cows.

The analytical control of some reproductive indicators of interest for decision-making at farm level, is a critical point for the implementation of the scientific knowledge in this field. Traditionally, the radio immunoassay (RIA) techniques have been used for the analysis of steroids in biological fluids. RIA was developed to determine insulin in human blood, and subsequently was implemented with new protocols to determine other hormones. These advances were crucial in Animal Reproduction, because provided information about the sexual hormonal profiles of different species. Nevertheless, the main disadvantage of RIA is to be a radioactive technique, and consequently special cautions should be taken for its routine use.[2] Therefore, in breeding programs, it would be interesting to have a more ecological and economical techniques for the analysis of hormonal and metabolic reproductive indicators. Near infrared (NIR) spectroscopy is being considered every day more as a powerful sensor for the analysis of biological fluids,[3] owing mainly to that is a very fast, inexpensive, versatile (multi-product and multi-constituent) and no-contaminant technique, moreover its easy management.

The present work shows the preliminary results obtained in a study aimed to the use of NIR for predicting progesterone levels in cattle plasma.




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## Material and methods

### Animals and samples collecting

Forty Holstein lactating cows, from a dairy farm located in south of Spain (38º02´N-4º10´W), were studied. These animals had an average of 3.2 services per conception, between three and ten years old and presented a good body condition. Oestrus detection was performed by pedometers (Westfalia, Germany).

Blood samples were taken from a coccygeal vessel into vacutainer tubes (sodium heparin). Each sample was centrifuged 10 min. (1000 g for 15 min.), and two aliquots plasma of each sample were stored at –20ºC until their analysis by RIA and NIR.

### Reference data

The RIA method in solid phase without extraction (Coat.A-Count, 125I, DPC, Spain) was used. This assay has been validated by cow plasma.[4] The intra- and inter-assay variation coefficients were 2.40% and 6.07%, respectively.

### Spectral data

Samples of cattle plasma were analysed using a Foss NIRSystems 6500 SY-I scanning monochromator fitted with a spinning module, working in reflectance mode in the spectral range 400–2500 nm. Measurements were made in folded-transmission gold reflector cam-lock cups, with a pathlength of 0.1 mm. Two spectra were measured per sample; the mean spectrum was used for subsequent chemometric analysis.

### Calibration development

Spectroscopic and chemical data were subjected to chemometric treatment using the software WinISI ver. 1.05 (Infrasoft International, Port Matilda, PA, USA).[5]

Modified partial least squares (MPLS) was the regression method used to develop the prediction equations, and the wavelength range selected was 1100–2500 nm (at 2 nm intervals). Several first and second derivative treatments and scatter correction methods were tested. The following statistical parameters were used to select the best calibration equations: standard error for the calibration set (*SEC*), standard error of cross-validation (*SECV*), coefficient of determination for the calibration process ($R^2$) or for the cross-validation process ($r^2$), and ratios *RPD* (DT·$SECV^{-1}$) and *RER* (Range·$SECV^{-1}$).[6]

## Results and discussion

Figure 1 show a typical spectrum of cattle plasma analysed by folded-transmission. As can be seen the traditional water bands are dominants in the plasma spectrum, as water is the main plasma constituent

The best NIR equation for prediction progesterone content in cattle plasma was developed using the first derivative treatment 1,4,4,1, and multiplicative scatter correction. The calibration statistics obtained are showed in Table 1.

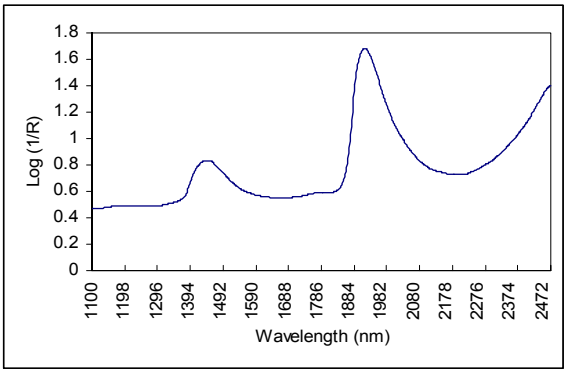


**Figure 1. NIR spectrum of cattle plasma.**

**Table 1. Calibration statistic for the prediction of progesterone level in cattle plasma (*n*=40).**

| Parameter | Mean | Range | *SD* | *SECV* | $r^2$ | *RPD* | *RER* |
|---|---|---|---|---|---|---|---|
| Progesterone Level | 1.47 | 0.05-5.47 | 1.41 | **0.75** | **0.73** | 1.88 | 7.23 |

There are not bibliographic references about the NIRS analysis of progesterone or other hormones in plasma or blood to compare the results obtained in the present paper. However, the Standard Error of Cross Validation compares very well with the Standard Error of the RIA method (0.62%). Therefore, it is known that in the clinical field, NIRS technology is being used in the analysis of different biological fluids to determine with high accuracy several constituents (glucose, deuterium, urea, albumin, etc).

These preliminary results show that the NIR calibration obtained may be used as a screening procedure of the progesterone content in cow plasma. Further work is in progress to improve the accuracy of the equation by different strategies (i.e. optimising sample presentation and increasing of samples of the calibration set, covering with homogeneity the variation range for this parameter).

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## References

1. G.E. Lamming and A.O. Darwash. *Anim. Reprod. Sci.* **52,** 175 (1998).
2. F.M. Van de Wiel and W. Koops. *Anim. Reprod. Sci.* **10,** 201 (1986).
3. E.W. Ciurczak, in *Near-Infrared Applications in Biotechnology*, Ed by R. Raghavachari. Marcel Dekker, Inc., New York, USA, p. 323 (2001).
4. C.C. Pérez, I. Rodríguez, F. España, M. Hidalgo, J. Dorado and J. Sanz, RIA de fase sólida para progesterona en plasma bovino, empleando calibradores humanos. VII Congreso Internacional de Medicina Bovina, Oviedo, España (2001).

5. ISI, The complete software solution using a single screen for routine analysis, robust calibrations, and networking. Manual. Foss NIRSystems/TECATOR. Infrasoft International, LLC. Sylver Spring MD, USA (2000).
6. P.C. Williams and D. Sobering, in *Near Infrared Spectroscoopy: The Future Waves*, Ed. By A.M.C. Davies and P.C. Williams. NIR Publications, Chichester, UK, p. 185 (1996).