September/October 1982 Volume 138, Number 5

British Veterinary Journal

Published since 1875

BAILLIÈRE TINDALL London

Br. vet. J. (1982). **138,** 436

DIRECT ENZYMEIMMUNOASSAY FOR DETERMINATION OF PROGESTERONE IN MILK FROM COWS

BY K.-I. ARNSTADT AND B. SCHMIDT-ADAMOPOULOU*

Institut für Physiologie der Süddeutschen Versuchs- und Forschungsanstalt für Milchwirtschaft, Technische Universität München, 8050 Freising-Weihenstephan, F.R.G.

SUMMARY

A direct enzymeimmunoassay for progesterone in whole and last milk (stripping) allows reliable detection of corpus luteum activity within one working day. The discriminatory level is 5 ng/ml.

INTRODUCTION

To avoid the disadvantages, mainly due to radioactivity, in radioimmunoassays enzymeimmunoassays (EIA) have been developed to measure progesterone in extracts of milk fat (Arnstadt, 1979; Arnstadt & Cleere, 1980, 1981), milk (van de Wiel, Kalis & Kamonpatana, 1982) and serum (Kamonpatana *et al.*, 1979; Nakao, 1980; Arnstadt, Grunert & Schulte, 1981). Direct estimation without extraction has been reported for cream from milk (Nakao & Kawata, 1980) and milk (Sauer, Foulkes & Cookson, 1981). The present report is of a direct estimation of progesterone with a commercially-available kit which enables assays to be made without a biochemically-specialized laboratory. A brief report has already been made (Arnstadt, 1982).

MATERIALS AND METHODS

The reagent and standard kit for enzymeimmunoassay of progesterone were obtained from Biolab, Munich, Germany, and NaCl, H_2O_2 and H_2SO_4 from Merck, Darmstadt, Germany. A PCP 6121 photometer with interface, calculator, printer and a special computer programme for EIA to print out the result directly in ng/ml milk was obtained from Co. Eppendorf, Munich, Germany.

* Guest from the Aristotelian University, Thessaloniki, Veterinary School, Dept. of Obstetrics, 12, Stavrou Voutyra St., Greece.

Enzymeimmunoassay

The standards contained 0·00, 0·03, 0·1, 0·3, 1·0 and 3·0 pmol/100 μl. The original standard solution contained 30 nmol/l and was diluted with double-distilled H_2O. Standards and samples were run in triplicate. To 100 μl standard 15 μl milk from oestrous cows was added. Sample tubes contained 100 μl H_2O instead of standard. One hundred μl antiserum suspension was added and incubated 30 min at 37°C and 10 min at 0°. After addition of 100 μl marker solution, the tubes were shaken (shaker: Edmund Bühler, SM, Germany) for 25 min at room temperature and for 30 min in ice. The unbound marker was removed by washing with 4 ml 0·15 M NaCl by centrifugation (2000 *g*, 5 min, 4°C). The washing was repeated with 4 ml 1 M NaCl. To the pellet 1 ml substrate solution (1 substrate-pill from Co. Biolab and 30 μl H_2O_2, 30%/55 ml H_2O) was added and incubated in the dark at room temperature for 1 h. The enzyme reaction was stopped by adding 0·25 ml 6 N H_2SO_4 and the extinction of the supernatant measured at 492 nm.

RESULTS

Direct estimation of 21 milk samples from days 0 and 6 of the oestrous cycle suggests that the discriminatory level of this EIA should be 5 ng/ml (Fig. 1). The discriminatory level is the same for whole milk or last milk (stripping); the higher fat content of last milk did not effect these results.

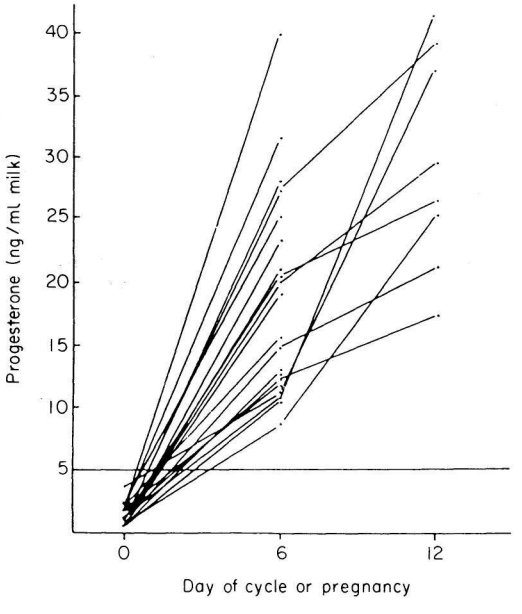

Fig. 1. Direct assay of progesterone with whole milk by enzymeimmunoassay (n = 21).

DISCUSSION

Some disadvantages in the direct determination of progesterone by EIA have already been reported. In the assay on cream from milk (Nakao & Kawata, 1980) the milk samples have to stand over-night for cream separation. Sauer *et al.* (1981) give two methods of direct EIA. The test tube EIA with a good standard curve did not deliver results the same day because of over-night incubation. The microtitre plate EIA had a standard curve too flat for a good discrimination between milk samples at the beginning of a cycle with low progesterone contents, i.e. between days 0 and 6.

The EIA reported here has several advantages. The reagents are commercially available (Biolab Company, Munich). The assay can be run in 4 to 5 h including handling time. The assay has a steep standard curve. It seems to provide a reliable discrimination between corpus luteum inactivity and activity at the beginning of the cycle, i.e. between day 0 and 6. Further results and extensive validation data will be published elsewhere.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance of Miss U. Mair and Miss G. Silberbauer.

REFERENCES

ARNSTADT, K. I. (1979). *Proceedings of the Conference on Applications of Radioimmunoassay and Related Methods in Animal Science, Warsaw,* ed. R. Stupnicki & B. Barcikowski.

ARNSTADT, K. I. (1982). *Zuchthygiene.* In press.

ARNSTADT, K. I. & CLEERE, W. F. (1980). *Zuchthygiene* **15,** 74.

ARNSTADT, K. I. & CLEERE, W. F. (1981). *Journal of Reproduction and Fertility* **62,** 173.

ARNSTADT, K. I., GRUNERT, E. & SCHULTE, B. (1981). *Zuchthygiene* **16,** 78.

KAMONPATANA, M., VAN DE WIEL, D. F. M., KOOPS, W., LEENANURUKSA, D., NGRAMSURIYAROJ, C. & USANAKORNKUL, S. (1979). *Theriogenology* **11,** 399.

NAKAO, T. (1980). *Acta endocrinologica* **93,** 223.

NAKAO, T. & KAWATA, K. (1980). *Proceedings of International Congress on Diseases of Cattle, Tel Aviv,* Vol. II, 916, ed. E. Mayer.

SAUER, M. J., FOULKES, J. A. & COOKSON, A. D. (1981). *Steroids* **38,** 45.

VAN DE WIEL, D. F. M., KALIS, C. H. J. & KAMONPATANA, M. (1982). *Proceedings of the CEC-Seminar on Factors Influencing Fertility in the Post-Partum Cow, Freising,* 1981, ed. H. Karg & E. Schallenberger. The Hague: M. Nijhoff.