POSSIBLE USE OF OVARIECTOMIZED EWES FOR ASSAY OF PASTURE OESTROGENS BY THE VAGINAL SMEAR (ALLEN-DOISY) METHOD

by D.R. LANG*, and D.R. LAMOND*

Summary

The possibility of using ovariectomized ewes for a vaginal smear (Allen-Doisy) assay for pasture oestrogens is being considered. Early problems associated with evaluation of the vaginal smear and priming are discussed.

I. INTRODUCTION

The possible use of ovariectomized ewes for oestrogen assay by the vaginal smear (Allen-Doisy) method has been investigated. The major advantage of vaginal smears over uterine weights is that the animals do not have to be killed for examination.

The initial investigations were:

(i) Feasibility of the vaginal smear for assaying pasture oestrogens, particularly the possibility of a 2 to 3 day assay.
(ii) Sensitivity and methods of increasing it.
(iii) Accuracy.
(iv) Possible use of a synthetic or natural oestrogenic steroid for use as a standard.

II. METHODOLOGY

(a) Collection of the Smear

The method is similar to that of Robinson and Moore (1956). Approximately 10 per cent. of the ewes had vaginal constrictions and were discarded.

(b) Staining Methods

Smears were air-dried and stained with methylene blue, or air-dried, fixed in methyl alcohol for a few minutes, and stained with Giemsa. Methylene blue gave variable results, particularly in staining leucocytes.

(c) Evaluation of Smears

Subjective methods of smear evaluation have proved successful in a wide range of mammals. Leucocytes disappear during oestrus, and cornified cells appear in large numbers during oestrus and metoestrus. The pattern is similar in the ewe, but rarely do leucocytes disappear entirely, and cornified cells are commonly seen at all stages of the cycle in smears obtained from ovariectomized ewes (Cole and Miller, 1935; Bell, Casida, and Darlow 1941; Robinson and Moore 1956; Radford and Watson 1955). As we were interested in detection and measurement of small amounts of oestrogen a concise definition of a positive (+ve) smear was necessary.

* Division of Animal Physiology, C.S.I.R.O., Pastoral Research Laboratory, Armidale, New South Wales.

(d) Importance of Priming

Different kinds and duration of priming were investigated, firstly because it was felt that there may be a method of priming which should give maximum sensitivity, and secondly because priming might assist evaluation of the smear.

III. RESULTS AND DISCUSSION

Factorial experiments were carried out in ovariectomized Merino ewes at 2 to 4 week intervals. When the experimental interval exceeded this period, consideration in evaluation of the smear was necessary. (See Moore and Robinson 1957 for discussion of this point.)

In the first two experiments, combinations of progesterone and oestrogen were given at various times prior to test doses of oestrogen. Smears were taken daily or twice daily, commencing on the day of the test dose, and in the experiment where a single priming dose was given 24 hours before the test dose, on that day also, and continued for 4 to 6 days. The principal conclusions from these experiments were:

(a) The duration of priming with progesterone does not influence the proportion of +ve smears. This agrees well with the general conclusions of Robinson, Moore, and Binet (1956).

(b) A single injection of 10 mg of progesterone one day before the test dose of oestrogen resulted in a higher proportion of cornified cells in relation to leucocytes, and hence a higher proportion of +ve's, than if 1 mg progesterone was given.

(c) Doses of stilboestrol in the range $0{\cdot}1$ - $1{\cdot}0\ \mu g$ greatly increased the number of leucocytes within 24 hours. The numbers were reduced within 48 hours and some cornified cells appeared, although it is difficult to distinguish the smears on Day 2 and subsequent days from those obtained from untreated ovariectomized ewes. With increasing doses of stilboestrol, leucocyte numbers increased within 24 hours, disappeared within 48 hours, and the typical +ve smear containing mostly cornified cells plus squamous cells followed.

The experimental design, together with the results of a recent 2^2 x 2 factorial experiment involving 64 ewes, is outlined in Table 1 and Fig. 1. The factors varied were:

(i) dose of progesterone — $1{\cdot}0, 10{\cdot}0$ mg,
(ii) dose of stilboestrol (priming) — $0{\cdot}1, 1{\cdot}0\ \mu g$,
(iii) test dose of stilboestrol — 10, 40 μg.

The progesterone and priming doses 'of stilboestrol were given on the one day and the test doses 24 hours later. Vaginal smears were taken on the day of the priming doses and thereafter until the third day after the test dose. Each smear was classified, firstly as to whether or not there were marked increases in leucocyte numbers, and secondly as to whether the smear constituted a positive on the basis outlined by Robinson and Moore (1956), i.e. marked increase in cornified cells and squamous cells together with disappearance of leucocytes. The smears from each ewe were examined in series, commencing with the Day 1 smear. The treatment each ewe received, however, was not known at the time of examination.

With 10 mg progesterone plus 1 μg stilboestrol on Day 1, satisfactory results for both the leucocyte picture and evaluation of smears were obtained. It is also possible that the sensitivity of the assay was greatest following this priming treatment.

The results suggest:

(i) that in assays where the normal positive cornified smears are to be considered, the priming dose should consist of approximately 10 mg progesterone plus 1 μg stilboestrol given on the day prior to the test dose.

(ii) that the rapid increase in leucocytes observed following low levels of stilboestrol should be examined as a possible basis for a sensitive assay.

IV. REFERENCES

BELL, T. D., CASIDA, L. E., and DARLOW, A. R. (1941).—Effects of oestrogen and progesterone upon the genital tract of the *ewe. Endocrinology 28:* 441.

COLE, H. H., and MILLER, R. F. (1935).—Changes in the reproductive organs of the ewe with some data bearing on their control. *Amer. J. Anat. 57: 39*

MOORE, N. W., and ROBINSON, T. J. (1957).—The vaginal response of the splayed ewe to repeated injections of oestradiol benzoate given alone or preceded by progesterone. *J. Endocrinol.* 14: 297.

RADFORD, H. M., and WATSON, R. H. (1955).—Changes in the vaginal contents of the Merino ewe throughout the year. *Aust. J. Agric. Res. 6: 43* 1.

ROBINSON, T. J., and MOORE, N. W. (1956).—The interaction of oestrogen and progesterone on the vaginal cycle of the *ewe. J. Endocrinol.* 14: 97.

ROBINSON, T. J., MOORE, N. W., and BINET, F. E. (1956).—The effect of duration of progesterone pretreatment on the response of the splayed ewe to oestrogen. *J. Endocrinol.* 14: 1.