

PLASMA PROGESTERONE CONCENTRATIONS DURING THE REPRODUCTIVE CYCLE OF EWES GRAZING YARLOOP CLOVER

J. M. OBST AND R. F. SEAMARK

*Department of Animal Physiology and Department of Obstetrics and Gynaecology,
University of Adelaide, Australia*

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Summary. Peripheral plasma progesterone concentrations were determined throughout the reproductive cycle of ewes grazing oestrogenic (Yarloop clover) pastures to provide an index of corpus luteum function. In ewes failing to conceive, luteal function declined after Day 13 and plasma progesterone concentrations had fallen to low 'oestrus' values by the 15th day after mating. In contrast, normal cyclic ewes grazing non-oestrogenic pastures maintained significant luteal function until the 15th day after mating. The mean periods between matings in both groups were, however, similar (17.5 and 17.1 days).

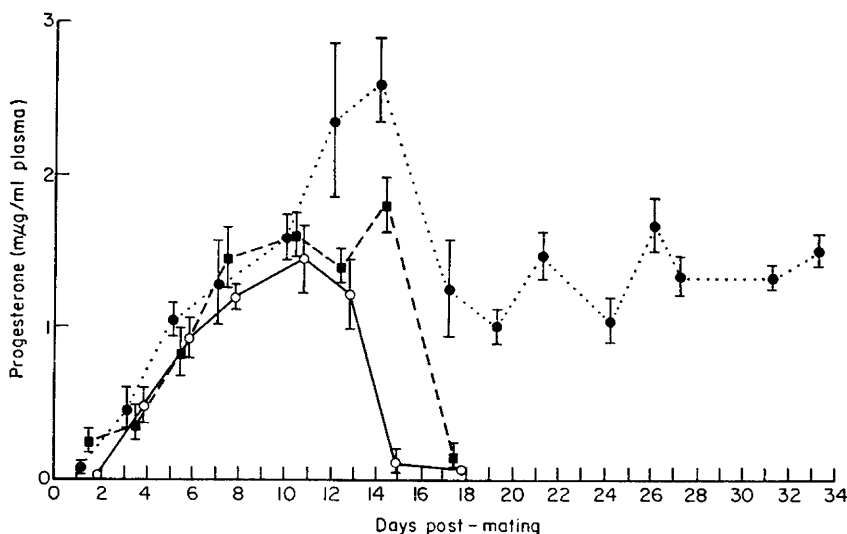
The failure of fertilization in ewes affected with Clover Disease has been attributed to a reduction in sperm transport or lowered sperm survival in the cervix and/or vagina (Turnbull, Braden & George, 1966; Lightfoot, Croker & Neil, 1967; Fels & Neil, 1968). The hormonal status of the affected ewes is unknown although cycle length and ovarian activity, as assessed by follicle development and ovulation rate, were not affected (Lightfoot *et al.*, 1967). Recent development of rapid and sensitive competitive protein-binding methods of hormone assay (Murphy, 1967) has made possible more direct assessment of ovarian endocrine function. The observations presented in this communication indicate that although oestrous cycle lengths of ewes grazing oestrogenic pastures may be normal, the period of corpus luteum function as determined by plasma progesterone concentration is reduced.

A hundred, Merino and Corriedale, 1½-year-old ewes on the Department of Agriculture Research Station at Parndana on Kangaroo Island were mated with four entire rams, fitted with sire-sine harnesses and crayons, on a germinating Yarloop clover pasture during February to March, 1969. Mating was recorded every 2nd day and the first six well-raddled ewes of each breed were bled by venipuncture every 2nd day for 35 days. The blood samples were centrifuged and the plasma frozen immediately after collection and stored at -14° C until assayed for progesterone.

Daily plasma samples collected from six ewes grazing pastures free of oestrogenic clovers were also assayed for progesterone using a competitive protein-binding technique after Murphy (1967). Oestrus in these ewes was

determined using a vasectomized ram. Laying-hen plasma was used as the source of corticosteroid-binding globulin and 1,2-T-cortisol as the tracer steroid. Plasmas (0.5 or 1.0 ml) were heated for 10 min at 60° C to denature any transcortin present and progesterone was extracted with 5 vol of petroleum ether (b.p. 40 to 60° C). Protein-bound steroid was separated on small Sephadex columns (Bassett & Hinks, 1969) and eluted directly into scintillation fluid. Method blanks and standard curves included an equivalent volume of wether or oophorectomized-ewe plasma.

Twenty-eight of the 100 ewes grazing Yarloop lambed compared to ninety-four of the 100 ewes of the same breeds on non-oestrogenic pasture. Of the twelve ewes mated on Yarloop and selected for progesterone sampling, six ewes conceived to the first mating and five of these subsequently lambed; lambing observations on the other ewe were uncertain and therefore results were deleted. The remaining six ewes (four Corriedale and two Merino) returned to service at least once and from these, nine cycles were followed for progesterone levels.



TEXT-FIG. 1. Plasma progesterone concentrations after mating of pregnant (●) and non-pregnant (○) ewes grazing oestrogenic pasture; non-pregnant ewes (■) grazing non-oestrogenic pasture. Each point represents the mean value (\pm S.E.M.) for at least five ewes.

The mean cycle length for ewes grazing oestrogenic pastures was 17.1 days (± 0.59 S.E.M.) and for non-oestrogenic pastures was 17.5 days (± 0.31 S.E.M.).

The patterns of progesterone concentration found in the ewes are shown in Text-fig. 1. Irrespective of oestrous-cycle length, the time of maintenance of corpus luteum function following mating, as measured by peripheral plasma progesterone, is 2 to 3 days shorter in infertile ewes grazing oestrogenic, compared to normal cyclic ewes on non-oestrogenic, pastures.

The progesterone concentrations of ewes grazing oestrogenic and non-oestrogenic pasture were similar up to Days 12 to 13 of the oestrous cycle, after which progesterone concentrations in the oestrogenic group continued to

fall to reach 'oestrus' levels at Days 14 to 15. However, those on the non-oestrogenic pasture rose to a second peak concentration at Days 14 to 15 followed by a fall to 'oestrus' levels by Days 17 to 18 of the cycle. At Days 14 to 15, the difference in mean progesterone concentration between ewes grazing oestrogenic and non-oestrogenic pasture was significant ($P < 0.001$). The pattern of progesterone concentration in the ewes grazing non-oestrogenic pasture is comparable to that described by Stabenfeldt, Holt & Ewing (1969) and it is also clear from their data that the same fall in progesterone occurs at Day 11, 12 or 13 of the cycle followed by a second peak of progesterone before 'oestrus' behaviour.

The hormonal mechanisms involved in this biphasic progesterone level and the apparent elimination of the second peak in ewes grazing oestrogenic pastures is currently being investigated. Recent findings by Karsch, Noveroske, Roche & Nalbandov (1969) suggest that follicular oestrogen contributes to the abrupt regression of the corpus luteum in normal cyclic ewes. It is, thus, possible that the ingested plant oestrogens may have a direct luteolytic effect on the corpus luteum or act indirectly by affecting follicular steroidogenesis or a uterine luteolytic agent.

In those animals which conceived whilst grazing oestrogenic pastures, the pattern of plasma progesterone concentration found in early pregnancy was similar to that for ewes on non-oestrogenic pasture. The characteristic rise in progesterone concentration in the late luteal phase is comparable to that found in the pregnant rhesus monkey and man (Neil, Johansson, Datta & Knobil, 1967; Johansson, 1969).

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