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PLASMA HORMONE LEVELS DURING PREGNANCY IN EWES GRAZING OESTROGENIC CLOVER

J. M. ORBST*, R. F. SEAMARK† and C. J. McGOWAN‡

Summary

Peripheral plasma hormone levels and reproductive performance of ewes grazing oestrogenic Yarloop clover have been compared with those of ewes grazing a non-oestrogen (grass) pasture on Kangaroo Island.

Ewe fertility (42 per cent) was significantly ($P < 0.001$) lower, and lamb mortality (50 per cent) significantly ($P < 0.001$) higher, on the Yarloop pasture than the comparable values of 88 per cent and 29 per cent respectively on grass pasture.

Mean plasma corticoid levels from the 120th to 145th day of gestation were higher, and progesterone levels from the 90th to 145th day were lower, in ewes grazing Yarloop than in ewes on grass pasture.

The possible physiological basis of the relationship of the observed hormonal disturbances to Clover Disease is discussed.

I. INTRODUCTION

The low reproductive rate in ewes is a continuing problem on Kangaroo Island pastures, initially established with oestrogenic subterranean clovers during the 1950's or early 1960's. Despite increasing soil fertility and attempts to reduce the dominance of these clovers in the pastures over the last 10 years, classical signs of Clover Disease (Bennetts, Underwood and Shier 1946) are still observed on some properties. Many properties, in addition, experience poor reproductive performance without the ewes necessarily exhibiting all the signs of Clover Disease. On these properties, the low ewe fertility and high lamb mortality underlying the failures may also be associated with selenium deficiency (Godwin, Kuchel, and Buckley 197 1; Obst unpublished), and adverse weather conditions at lambing (Obst and Day 1968).

'Recent studies have revealed endocrinological disturbances in sheep grazing oestrogenic pastures on Kangaroo Island (Obst and Seamark 1970; Obst, Seamark, and McGowan 1971). This paper reports further measurements of pregnancy hormones in the ewe in relation to parturition and lamb survival.

*Departments of Animal Physiology and †Obstetrics and Gynaecology, University of Adelaide, Adelaide and ‡Department of Agriculture, Research Centre, Parndana, Kangaroo Island, South Australia.

II. MATERIALS AND METHODS

(a) Experimental animals and management

One hundred South Australian Merino and 100 Corriedale ewes, 1½ years old were divided on criteria of breed and liveweight into two groups. One group grazed a Yarloop dominant (> 80%) pasture and the other a pasture based on perennial rye grass. Changes in size of the bulbo-urethral gland of wethers grazed with the ewe groups confirmed the oestrogenic activity of the Yarloop pasture and the non-oestrogenic quality of the grass pasture.

Mating for 6 weeks with 4 per cent rams fitted with Sire-sine harness and crayons began in mid-February of 1969 and 1970. Following the 1970 mating, pregnant ewes (5 Merino and 5 Corriedale from each pasture) were selected for weekly blood sample collection throughout pregnancy.

Lambing observations were made twice daily and each lamb was identified with its mother soon after birth. A post-mortem examination was made on ewes which died near term, or were unable to lamb even with manual assistance, to ascertain the number of dead lambs *in utero.*

TABLE 1

Reproductive performance of ewes ***grazing Yarloop, or rye grass pasture***

Yarloop pasture	*Merinos*		*Corriedales*		*Total*
	1969	1970	1969	1970	
Ewes mated	50	43	50	44	187
Fertile ewes	20	29	7	22	78
% Fertile ewes	40	67	14	50	42
Lambs born	20	34	7	27	88
Lamb deaths	11 (0)	12 (4)	3 (0)	18 (6)	44 (10)
% Lamb mortality†	55	35	43	67	50
Grass pasture					
Ewes mated	52	46	48	38	184
Fertile ewes	47	34	48	33	162
% Fertile ewes	90	74	100	87	88
Lambs born	50	44	49	41	184
Lamb deaths	16 (0)	17 (4)	13 (0)	7 (0)	53 (4)
% Lamb mortality	32	39	27	17	29
Differences (χ^2) between pastures					
(i) Fertile ewes	***	ns	***	***	***
(ii) Lamb deaths	ns	ns	—	***	***

***($P < 0.001$) ns ($P > 0.05$).

Figures in parenthesis indicate the number of lambs that died *in utero* and were recovered post mortem.

†Lamb mortality % = $\left(\frac{\text{Lamb deaths}}{\text{Lambs born}} \times \frac{100}{1}\right)$ where lambs born = total number of lambs born dead or alive or recovered in ***utero.***

Lamb deaths = total number of lamb deaths up to 4 weeks of age including lambs recovered ***in utero.***

(b) ***Hormone*** *assays*

Serial peripheral plasma samples from ewes on each pasture were assayed simultaneously for progesterone as described by Obst and Seamark (1970), except that dog plasma and not hen plasma was used as the source of binding protein. Progesterone concentrations were expressed in ng/ml plasma. Corticoid concentrations were determined by a method similar to that of Bassett and Hinks (1969). Plasma corticoid concentrations, expressed as cortisol equivalents in ng/ml were corrected to allow for interference from plasma progesterone present in the ethanol extract.

III. RESULTS

The reproductive performance of ewes grazing Yarloop or grass pasture is shown in Table 1. Fertility (42 per cent) while grazing Yarloop was significantly ($P < 0.001$) lower than the fertility (88 per cent) of ewes grazing the grass pasture. Total lamb mortality on Yarloop (50 per cent) was significantly ($P < 0.001$) higher than the mortality on grass pasture (29 per cent). The net reproductive rate defined as the number of lambs surviving to marking from every 100 ewes mated was 23.5 on the Yarloop pasture and 7 1.2 on the grass pasture.

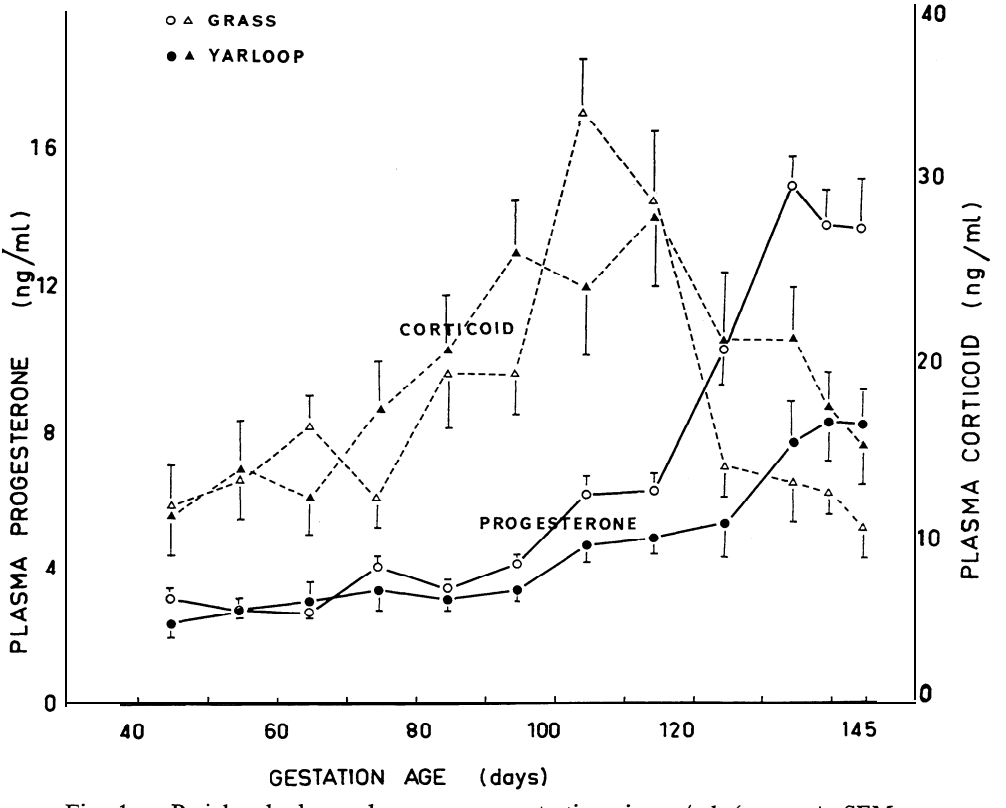

Fig. 1. — Peripheral plasma hormone concentrations in ng/ml (mean ± SEM: n = 9 or 10) in ewes grazing a grass or Yarloop pasture.
Progesterone — ○ — ○, grass pasture; ● — ● Yarloop pasture
Corticoid — △ -- △, grass pasture; ▲ -- ▲ Yarloop pasture

The mean plasma hormone levels and patterns within each pasture were similar for both breeds and are therefore combined (Figure 1). Mean plasma progesterone levels were siginficantly ($P < 0.05$ — < 0.001) lower in ewes grazing Yarloop than in ewes on grass pasture from the 90th day of gestation to term.

On both pastures, plasma corticoid levels increased significantly ($P < 0.001$) from day 40 to a maximum value at 100-120 days gestation; between 120 and 140 days the levels decreased, but in the ewes on Yarloop the mean plasma corticoid concentration remained significantly ($P < 0.05$) higher than in those grazing the grass pasture.

IV. DISCUSSION

Fertility was halved among those ewes grazing Yarloop clover, especially during the first mating on germinating Yarloop in 1969. Mating in 1970 took place while the ewes fed on dry Yarloop residues supplemented with Yarloop clover hay; fertility increased, but it was still lower, especially in the Corriedales, than the fertility of ewes on the grass pasture. These results suggest that mating on green Yarloop induced a temporary infertility in some ewes, while others remained infertile in 1970 following 9 months grazing on green Yarloop.

Determinations of plasma progesterone concentrations throughout the oestrous cycle of the young ewes grazing oestrogenic pasture suggested that corpus luteum function in the infertile animals was reduced (Obst and Seamark 1970). Similar determinations made during the 1970 mating period (Obst and Seamark unpublished) indicated a higher proportion of ewes with normal ovarian function, which was reflected in the improved fertility.

The observations presented above on pregnant animals reveal further endocrinological disturbances in ewes grazing oestrogenic pastures, particularly in late pregnancy when there are significantly lowered plasma progesterone levels and higher corticoid levels in the ewes grazing Yarloop compared with those on grass pasture. These results also suggest that an inverse relationship exists between plasma progesterone and corticoid.

Factors which regulate the concentration of progesterone or corticoid in the plasma at any one time are only partially understood. The increasing amounts of progesterone in the maternal circulation during the latter half of pregnancy are mainly of placental origin (Mattner and Thorburn 1971) , whereas the origin of the corticoid is most probably the maternal adrenal (Paterson and Harrison 1968). Present concepts of endocrine control of pregnancy visualise the foetal-placental unit as being autonomous. If the differences in plasma progesterone reflect differences in hormone production rate between sheep grazing oestrogenic and non-oestrogenic pastures, the plant oestrogens or their metabolites could. affect placental hormone synthesis. Alternatively, the differences may reflect changes in metabolic clearance rates or differences in distribution within the maternal or foetal components e.g. the decrease in maternal plasma corticoid during the latter part of pregnancy also observed by Saba (1965) and Paterson and Harrison (1967, 1968) has been attributed to an expansion in plasma volume, or alter-

natively an increase in foetal metabolism, as maternal corticoid will cross the placenta (Dixon et *al.* 1970). Thus the higher maternal corticoid in sheep grazing Yarloop may reflect an inhibition of or reduction in foetal metabolism of corticoid.

Since a functioning foetal pituitary-adrenal axis appears necessary for successful parturition (Liggins, Kennedy, and Holm 1967; Drost and Holm 1968) and lamb survival, it is postulated that the high maternal corticoid in late pregnancy suppressed foetal adrenal function, causing both the prolonged parturition and higher mortality of lambs observed in the present study. Alternatively, the low plasma progesterone concentrations, which lead to an abnormally high plasma oestrogen : progesterone ratio during pregnancy in ewes grazing Yarloop (Obst and Seamark unpublished), may prevent the normal pre-partum dilatation of the cervix. Thus even though uterine contractions are initiated, the foetus could not distend the contracted cervix and lamb death *in utero* is inevitable. The terminal sign of this sequence of events would be uterine inertia.

It is concluded that the study of endogenous plasma hormone levels to elucidate the complex mode of action of ingested phyto-oestrogens is a useful experimental approach, and may lead to a better understanding of Clover Disease.

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