

Influence of Haemoglobin Type and Selenium Status on Peripheral Plasma Progesterone and Corticosteroid Concentration in Ewes Grazing Oestrogenic Pastures

S. K. Walker, J. M. Obst,^A D. H. Smith, G. P. Hall^B and P. F. Flavel

Department of Agriculture and Fisheries, G.P.O. Box 1671, Adelaide, S.A. 5001.

^A Present address: Animal Research and Development Centre, P.O. Box 123, Bogor, West Java.

^B Present address: Division of Entomology, CSIRO, P.O. Box 1700, Canberra, A.C.T. 2601.

Abstract

Peripheral plasma progesterone and corticosteroid concentrations were studied in ewes grazing oestrogenic pastures during oestrus, pregnancy and parturition in 1974 and during pregnancy in 1975. Haemoglobin (Hb) type A ewes had significantly higher mean corticosteroid concentrations than HbB ewes in both years. In 1974 the concentration of progesterone did not differ significantly between Hb types whilst in 1975 the concentration was significantly ($P < 0.001$) higher in HbA ewes.

The mean corticosteroid concentrations were higher in selenium-supplemented ewes of both Hb types than in unsupplemented ewes in 1974 but lower in 1975. Selenium treatment did not significantly influence the progesterone concentration in ewes of both Hb types in 1974 but significantly ($P < 0.01$) increased it in 1975.

It is concluded that the higher concentration of plasma corticosteroids or factors regulating the relationship between corticosteroids and progesterone could be responsible for the higher estimates of embryonic mortality reported in HbA ewes.

Introduction

Both haemoglobin (Hb) type and selenium have been associated with the reproductive performance of sheep grazing oestrogenic pastures. It was reported that the HbA gene, under these circumstances, was associated with fertility (Obst *et al.* 1971) and that administration of selenium reduced the number of lambs born to HbA and AB ewes compared to the number born to HbB ewes (Obst *et al.* 1974). Results of Walker (1977) indicated that in flocks grazing oestrogenic pastures HbA ewes had a higher estimate of embryonic mortality than HbB ewes. On non-oestrogenic pastures the HbB gene has been associated with fertility (Evans and Turner 1965). However, the relationship between Hb type and selenium status has not been investigated in sheep grazing non-oestrogenic pastures.

Several anomalies arise as a result of ewes grazing oestrogenic pastures. Luteal function may be shortened during oestrus (Obst and Seamark 1975) and compared with ewes grazing non-oestrogenic pastures, there is a lower concentration of plasma progesterone in the later half of pregnancy (Obst and Seamark 1975) and a higher concentration of corticosteroids after 120 days gestation (Obst *et al.* 1972). The significance of these differences in relation to ewe fertility is unknown.

In the experiment reported here the concentrations of peripheral plasma progesterone and corticosteroids were monitored in HbA and HbB ewes grazing oestrogenic pastures. The influence of the selenium status of the ewes on these concentrations is also reported.

Materials and Methods

In total 110 South Australian strong-wool Merino (Bungaree) ewes, consisting of equal numbers of HbA and HbB types, grazed pastures dominated by Yarloop clover (*Trifolium subterraneum* cv. Yarloop) at Kangaroo Island Research Centre from 1972 to 1975 inclusive. The ewes were 1.5 years of age at the commencement of the grazing treatment. Half the ewes of each Hb type were randomly allocated to a selenium treatment. These ewes received 25 mg of selenium orally as sodium selenite 1 month before mating, after mating, at mid-pregnancy and pre-lambing in each year. Samples of blood were taken from randomly selected ewes at these times to monitor selenium concentrations for which the fluorimetric technique of Watkinson (1966) was used. Detailed mating and lambing records were taken over the 4-year period. The content of Yarloop clover in the pasture was estimated on a dry matter basis in each year and pasture oestrogenicity was assessed by isoflavone determination and bulbo-urethral gland enlargement (Obst and Seamark 1975). Peripheral plasma progesterone and corticosteroid concentrations were monitored in 1974 and 1975 following 2 years of depressed fertility in the ewes.

1974 Determinations

Peripheral plasma hormone concentrations were studied during oestrus, early pregnancy (0–48 days) and mid-late pregnancy (48 days to parturition). In February, daily blood sampling of 32 ewes (eight from each treatment group, namely HbA–Se, HbA+Se, HbB–Se, and HbB+Se) commenced on the day mating to a vasectomized ram was observed (day 1). Approximately 15 ml of venous blood per ewe was collected at 0900 h on each day of the oestrous cycle; the blood was centrifuged and the plasma frozen. These ewes were mated to entire rams at the next oestrus and blood samples were collected in a similar manner three times per week (Monday, Wednesday and Friday) until 48 days after mating. Half of the 32 ewes conceived.

Sixteen ewes, whose mating records indicated pregnancy, were selected from the remainder of the flock to replace the ewes which did not conceive. During the remainder of pregnancy the blood of all ewes was sampled mid-weekly at 0900 h until 8 days before expected parturition, when daily sampling commenced. Samples were taken at 0800, 1600 and 2400 h from 2 days before to 2 days after parturition.

1975 Determinations

Peripheral plasma progesterone and corticosteroids were monitored during pregnancy. Following mating, eight ewes from each treatment group were randomly selected from those ewes thought to be pregnant (on their mating records). Blood samples were taken weekly from the fifth week of pregnancy until the week of expected parturition.

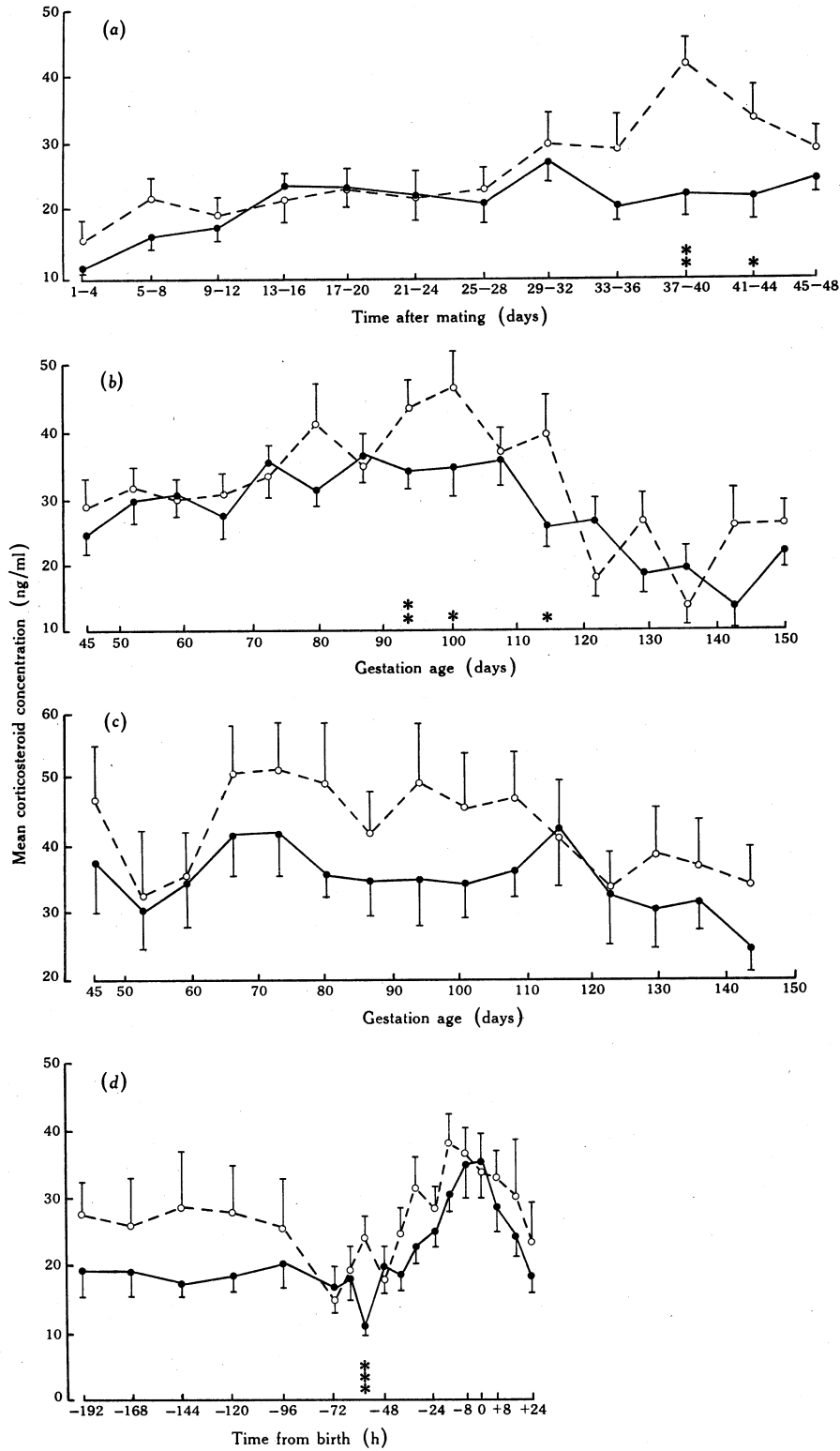
Hormone Assays

Progesterone was assayed by the competitive protein binding procedure described by Thorburn *et al.* (1969) and Obst and Seamark (1975). Dog plasma was the source of the globulin for binding of the corticosteroids. Standards containing 0, 0.2, 0.4, 1.0, 2.0 and 4.0 mg of progesterone added to 0.5 ml of wether plasma were run with each assay. A coefficient of variation of 5.12% was obtained. Corticoids were assayed by the competitive protein binding procedure described by Obst (1971). This method gave a coefficient of variation of 5.24%.

Statistical Analysis

Results were subjected to analysis of variance and Student's *t*-test. Hormone data of early pregnancy were analysed within 4-day periods.

Fig. 1. Influence of Hb type on the mean peripheral plasma corticosteroid concentration (a) during early pregnancy in 1974, (b) during mid-late pregnancy in 1974, (c) during mid-late pregnancy in 1975, and (d) at parturition in 1974. ○ HbA ewes ($n = 12$). ● HbB ewes ($n = 13$, except in c when $n = 12$). Standard errors are shown by vertical lines. * $0.01 < P < 0.05$. ** $0.001 < P < 0.01$. *** $P < 0.001$.



Results

The mean Yarloop clover content of pastures grazed in 1974 and 1975 was 54.4% and the content of formononetin, genistein and biochanin A in the Yarloop clover in 1975 was 1.14, 0.86 and 0.21% respectively. Bulbo-urethral gland scores of 2 or 3 were recorded in all wethers in each year. The mean concentrations of selenium in whole blood samples were 0.43 ± 0.02 , 1.21 ± 0.07 , 0.39 ± 0.02 , and 1.20 ± 0.04 $\mu\text{mol/l}$ for the HbA-Se, HbA+Se, HbB-Se and HbB+Se groups respectively. There were no significant interactions between Hb type and selenium treatment in either the progesterone or corticosteroid data.

Table 1. Mean plasma corticosteroid and progesterone concentration (\pm s.e.) in HbA and HbB types and selenium-treated (+Se) and unsupplemented (-Se) ewes at various stages from oestrus to parturition

All values are for 1974 except where shown otherwise. Hb types were pooled for selenium treatment groups

Stage of pregnancy	Corticosteroid concn (ng/ml)		Progesterone concn (ng/ml)	
	HbA	HbB	HbA	HbB
Oestrous cycle	13.4 ± 0.6	12.0 ± 0.6	1.66 ± 0.11	1.95 ± 0.14
Early pregnancy	24.9 ± 1.2	$20.9 \pm 0.8^{**}$	2.55 ± 0.15	2.57 ± 0.14
Mid-late pregnancy				
1974	31.8 ± 1.1	$27.4 \pm 0.8^{**}$	4.29 ± 0.18	4.39 ± 0.23
1975	42.6 ± 1.8	$35.8 \pm 0.8^{**}$	7.03 ± 0.32	$5.43 \pm 0.22^{***}$
Parturition	26.4 ± 1.1	$20.7 \pm 0.8^{***}$	4.85 ± 0.29	$5.66 \pm 0.27^*$
	-Se	+Se	-Se	+Se
Oestrous cycle	12.2 ± 0.7	13.3 ± 0.6	1.82 ± 0.11	1.71 ± 0.11
Early pregnancy	21.2 ± 0.9	23.6 ± 1.0	2.49 ± 0.16	2.63 ± 0.13
Mid-late pregnancy				
1974	27.8 ± 1.0	$31.4 \pm 1.0^*$	4.33 ± 0.23	4.32 ± 0.21
1975	40.6 ± 1.8	37.8 ± 1.5	6.76 ± 0.31	$5.68 \pm 0.25^{**}$
Parturition	23.0 ± 0.9	23.5 ± 0.9	5.33 ± 0.31	5.06 ± 0.25

* $0.01 < P < 0.05$.

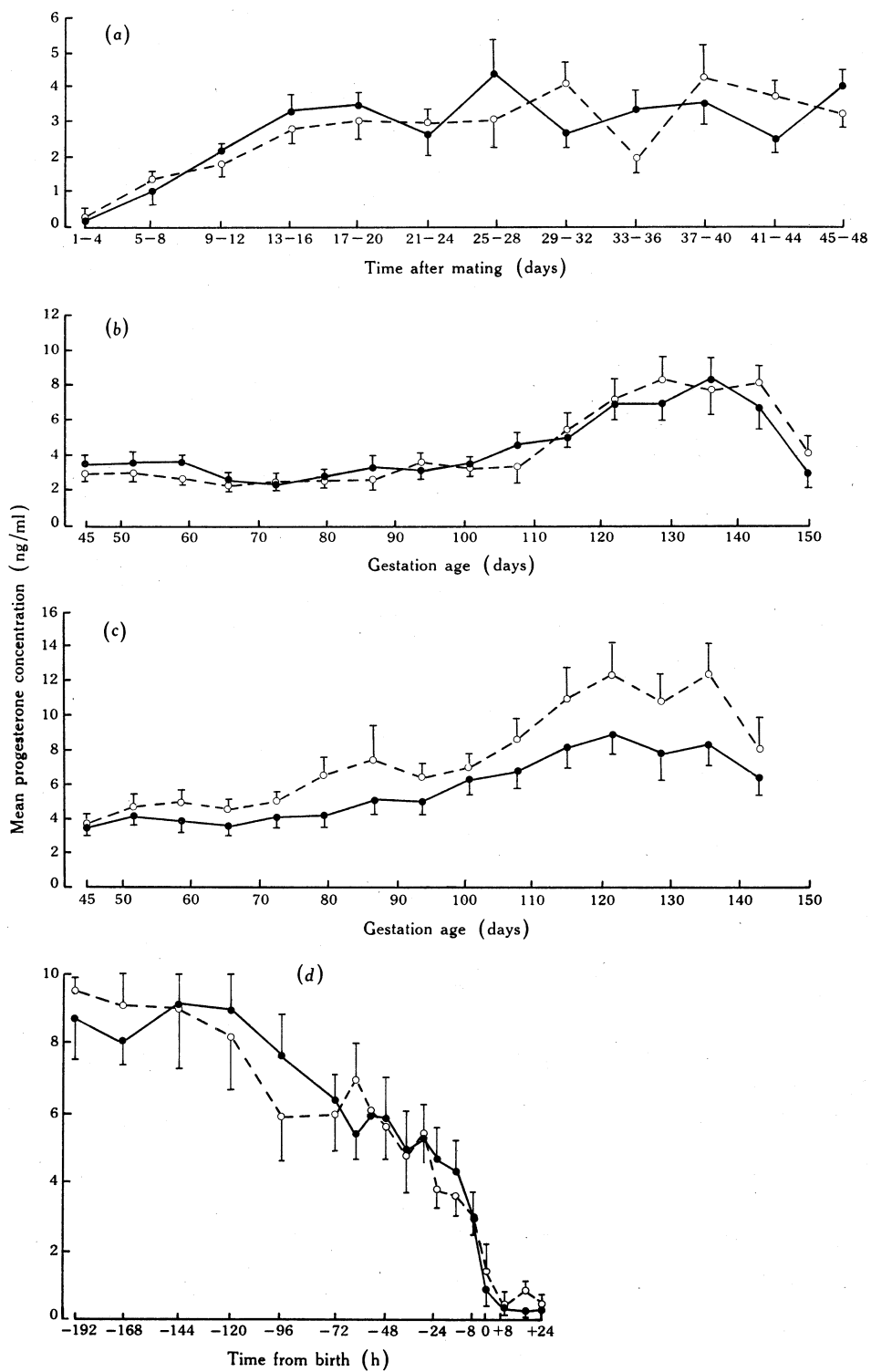
** $0.001 < P < 0.01$.

*** $P < 0.001$.

Hb Types

Mean corticosteroid concentration in HbA ewes was higher than in HbB ewes during oestrus (n.s.), early pregnancy ($P < 0.01$), mid-late pregnancy ($P < 0.01$) and parturition ($P < 0.001$) in 1974 and it was again significantly higher ($P < 0.01$) in HbA ewes than in HbB ewes during mid-late pregnancy in 1975 (Table 1). Consistent differences in the corticosteroid concentration between Hb types began to appear at about 25–28 days after mating (Fig. 1a) in 1974, significant differences occurring on days 37–40 ($P < 0.01$) and days 41–44 ($P < 0.05$). During mid-late pregnancy differences were most apparent between days 90 and 115 in 1974 (Fig. 1b)

Fig. 2. Influence of Hb type on the mean peripheral plasma progesterone concentration (a) during early pregnancy in 1974, (b) during mid-late pregnancy in 1974, (c) during mid-late pregnancy in 1975, and (d) at parturition in 1974. ○ HbA ewes ($n = 12$). ● HbB ewes ($n = 13$, except in c when $n = 12$). Standard errors are shown by vertical lines.



and days 65 and 115 in 1975 (Fig. 1c). As parturition approached in 1974 corticosteroid concentration increased 16 h earlier in HbA ewes than in HbB ewes and then declined 16 h before birth whereas the decline in HbB ewes did not take place until birth had occurred (Fig. 1d).

In 1974, the mean progesterone concentration was lower in HbA ewes than in HbB ewes during oestrus (n.s.), early pregnancy (n.s.), mid-late pregnancy (n.s.) and parturition ($P < 0.05$) (Table 1; see also Figs 2a-d); the latter difference occurred between -144 and -72 h (Fig. 2d). On the other hand, HbA ewes had consistently higher concentrations of progesterone than HbB ewes during pregnancy in 1975 (Fig. 2c). The mean result (Table 1) was significantly different ($P < 0.001$). The differences recorded during pregnancy in both years were not confounded by differences in fecundity.

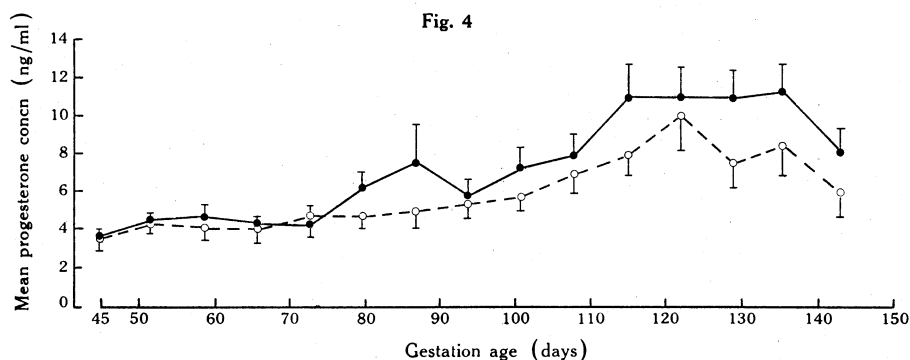
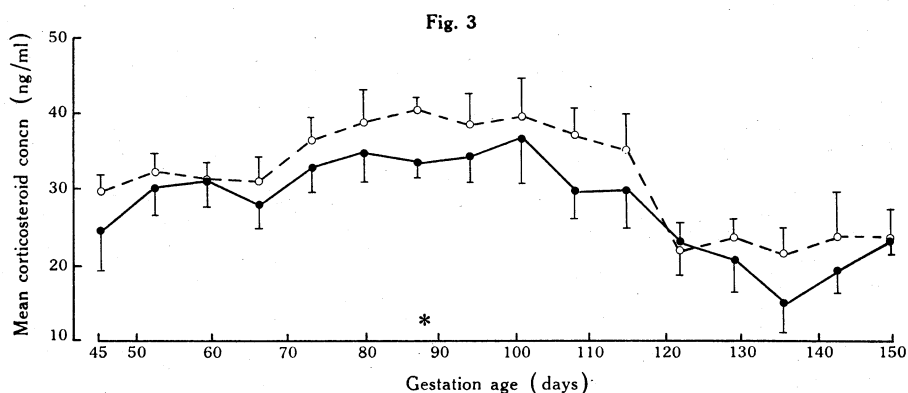


Fig. 3. Influence of selenium supplementation on the mean peripheral plasma corticosteroid concentration during mid-late pregnancy in 1974. \circ Selenium supplemented ewes ($n = 12$). \bullet Un-supplemented ewes ($n = 13$). Standard errors are shown by vertical lines. * $0.01 < P < 0.05$.

Fig. 4. Influence of selenium supplementation on the mean peripheral plasma progesterone concentration during mid-late pregnancy in 1975. \circ Selenium supplemented ewes ($n = 12$). \bullet Un-supplemented ewes ($n = 12$).

Selenium

In 1974, ewes of both Hb types which received selenium had a higher mean corticosteroid concentration than unsupplemented ewes during oestrus (n.s.), early pregnancy (n.s.), mid-late pregnancy ($P < 0.05$) and parturition (n.s.) (Table 1).

The corticosteroid concentrations during mid-late pregnancy are shown in Fig. 3. Significant differences were not obtained during pregnancy in 1975 (Table 1).

Selenium supplementation did not significantly influence the mean progesterone concentration in ewes of both Hb types in 1974 (Table 1). In 1975, the selenium-treated ewes had a significantly ($P < 0.01$) lower mean concentration of progesterone during mid-late pregnancy than the control ewes (Table 1); differences were apparent throughout most of pregnancy (Fig. 4).

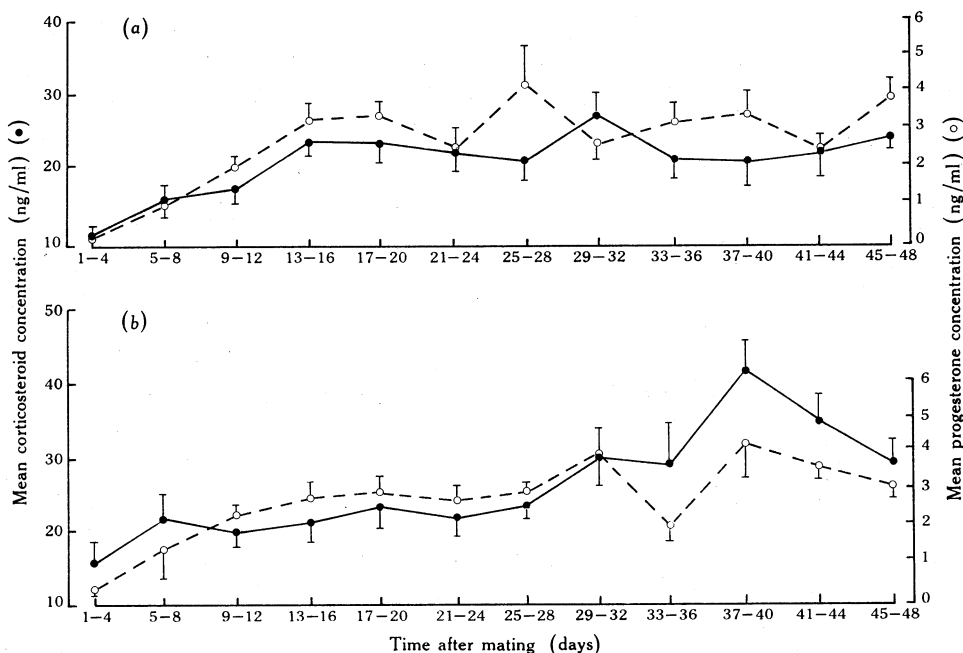


Fig. 5. Relationship between mean peripheral plasma progesterone (○) and corticosteroid (●) concentration in (a) 13 HbB ewes and (b) 12 HbA ewes during early pregnancy in 1974.

Discussion

In ewes that grazed oestrogenic pastures the HbA type had higher concentrations of plasma corticosteroids than did the HbB type. Whether this higher concentration in HbA ewes is characteristic of flocks grazing oestrogenic pastures remains to be investigated. It does, however, provide a physiological basis on which one difference in reproductive ability between Hb types can be explained. Evidence of Walker (1977) collected from nine flocks grazing oestrogenic pastures indicated that the estimate of embryonic mortality (Dolling and Nicolson 1967) in the South Australian strong-wool Merino was higher in HbA sheep than in HbB sheep. In the study reported in this paper the estimate of embryonic mortality was 4.4% higher (n.s.) in HbA ewes than in HbB ewes. Furthermore, the results of Arora *et al.* (1971) and Meyer *et al.* (1967) for sheep grazing non-oestrogenic pastures indicate similar findings.

Differences in corticosteroid concentrations during pregnancy first occurred 25–28 days after mating (Fig. 1a). Wroth and Lightfoot (1976) reported that in flocks grazing oestrogenic pastures approximately 50% of embryo loss occurred sufficiently late to delay the return to service for 26–42 days. Thus the significant

differences in the corticosteroid concentrations occurred at times when an increase in the estimate of embryonic mortality of HbA ewes could be expected.

Obst (1971) and Obst *et al.* (1972) reported an inverse relationship between plasma corticosteroid and progesterone concentrations in sheep, and Obst (1971) postulated that corticosteroids were involved in the control of progesterone synthesis. This relationship was apparent during pregnancy in our study. However, during early pregnancy (0–48 days) it was more apparent in the HbB group than in the HbA group (Fig. 5). Hence it is feasible that the higher estimate of embryonic mortality found in HbA ewes resulted not from the high concentration of corticosteroids *per se* but from a disturbance of the mechanisms controlling the relationship between plasma progesterone and corticosteroids.

High corticosteroid concentrations during pregnancy are a feature of ewes grazing oestrogenic pastures (Obst *et al.* 1972). Obst and Seamark (1975) reported an association between the length of parturition and the concentration of plasma corticosteroids in the 8-h period before birth. Obst (1971) postulated that a high concentration of maternal corticosteroids may inhibit the development of the foetal pituitary–adrenal axis necessary for successful parturition (Liggins *et al.* 1967; Drost and Holm 1968). There was no evidence in our study that the difference in corticosteroid concentrations between HbA and HbB ewes affected lambing. The incidence of dystocia was only slightly higher in the HbA ewes (2.9%) than in the HbB ewes (2.6%) and the mean gestation lengths did not differ.

There appears to be no previous evidence to indicate that selenium supplementation affects endocrine function. The significance of the increase in the concentration of corticosteroids obtained in 1974 is difficult to assess particularly as selenium supplementation is reported to reduce embryonic mortality (Hartley 1963; Mudd and Mackie 1973). In addition, the result was not repeated in 1975, although in that year selenium-treated ewes had a significantly ($P < 0.01$) lower concentration of plasma progesterone than the untreated ewes. Further investigation of this topic is warranted.

Acknowledgments

The assistance of the staff of Kangaroo Island Research Centre, Mesdames Fay Bradley, Leona Coleman, Jane Hawthorne and Mr C. H. S. Dolling, is appreciated. Dr G. J. Judson conducted the selenium analyses and the University of Western Australia determined the isoflavone contents of clover. This investigation was financially supported by the Wool Research Trust Fund.

References

- Arora, C. L., Acharya, R. M., and Kakar, S. N. (1971). A note on the association of haemoglobin types with ewe and ram fertility and lamb mortality in Indian sheep. *Anim. Prod.* **13**, 371–3.
- Dolling, C. H. S., and Nicolson, A. D. (1967). Vital statistics for an experimental flock of Merino sheep. IV. Failure in conception and embryonic loss as causes of failure to lamb. *Aust. J. Agric. Res.* **18**, 767–88.
- Drost, M., and Holm, L. W. (1968). Prolonged gestation in ewes after foetal adrenalectomy. *J. Endocrinol.* **40**, 293–6.
- Evans, J. V., and Turner, H. N. (1965). Haemoglobin type and reproductive performance in Australian Merino sheep. *Nature (London)* **207**, 1396–7.
- Hartley, W. J. (1963). Selenium and ewe fertility. *Proc. N.Z. Soc. Amin. Prod.* **23**, 20–7.

- Liggins, G. C., Kennedy, P. C., and Holm, L. W. (1967). Failure of initiation of parturition after electrocoagulation of the pituitary of the fetal lamb. *Am. J. Obstet. Gynecol.* **98**, 1080-6.
- Meyer, H., Lohse, B., and Gröning, M. (1967). A contribution to haemoglobin and blood potassium polymorphism in the sheep. *Anim. Breed Abstr.* **36**, No. 1550.
- Mudd, A. J., and Mackie, I. L. (1973). The influence of vitamin E and selenium on ewe prolificacy. *Vet. Rec.* **93**, 197-9.
- Obst, J. M. (1971). Hormone studies on ewes grazing Yarloop clover. Ph.D. Thesis, University of Adelaide.
- Obst, J. M., McGowan, C. J., and Walker, S. K. (1974). Interactions between selenium and haemoglobin type in the reproductive performance of ewes grazing oestrogenic pasture. *Proc. Aust. Soc. Anim. Prod.* **10**, 135.
- Obst, J. M., and Seamark, R. F. (1975). Hormone studies on ewes grazing an oestrogenic (Yarloop clover) pasture during the reproductive cycle. *Aust. J. Biol. Sci.* **28**, 279-90.
- Obst, J. M., Seamark, R. F., and McGowan, C. J. (1971). Haemoglobin type and fertility of Merino ewes grazing oestrogenic (Yarloop clover) pastures. *Nature (London)* **232**, 497-8.
- Obst, J. M., Seamark, R. F., and McGowan, C. J. (1972). Plasma hormone levels during pregnancy in ewes grazing oestrogenic clover. *Proc. Aust. Soc. Anim. Prod.* **9**, 204-8.
- Thorburn, G. D., Bassett, J. M., and Smith, I. D. (1969). Progesterone concentration in the peripheral plasma of sheep during the oestrous cycle. *J. Endocrinol.* **45**, 459-69.
- Walker, S. K. (1977). Studies on the interrelations of phyto-oestrogens, haemoglobin type and selenium in the sheep. M.Ag.Sc. Thesis, University of Adelaide.
- Watkinson, J. H. (1966). Fluorimetric determination of selenium in biological material with 2,3-diaminonaphthalene. *Analyt. Chem.* **38**, 92.
- Wroth, R. H., and Lightfoot, R. J. (1976). A study of reproductive wastage among commercial sheep flocks grazing oestrogenic pastures in south Western Australia. *Proc. Aust. Soc. Anim. Prod.* **11**, 225-8.

