# INFLUENCE OF LUPIN FEEDING BEFORE AND AFTER JOINING ON PLASMA PROGESTERONE AND FERTILITY IN MERINO EWES

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## SUMMARY

Merino ewes *(n* = 300) grazing on sparse annual pasture and supplemented with hay were given a CIDR (controlled internal drug release) device to synchronise oestrus. Ewes in Groups 1 and 2 received 750 g/head of lupin grain each day for 9 days prior to the expected oestrus and ewes in Group 3 received a hay-only supplement. After joining ewes in Group 1 continued to receive the lupin supplement while ewes in Groups 2 and 3 received a hay-only supplement. Ewes were examined by endoscope on days 6 and 7 after mating to determine the number of *corpora lutea* (CL) and half of the ewes in each group were given a CIDR device on day 10. All ewes were blood sampled for plasma progesterone on day 12. CIDRs were removed on day 14 and returns to oestrus were detected. Ewes were ultrasonically scanned on days 65-66 when pregnancy was confirmed and foetal numbers were determined.

Ewes fed lupins prior to joining had a significantly higher ovulation rate than those fed hay only (1.58 *v.* 1.46 ovulations per ewe ovulating, $P < 0.05$). Within ovulation class (1 or 2 + CL), mean peripheral plasma progesterone concentrations in ewes fed lupins after mating were significantly *(*$P < 0.05$) reduced compared with those fed hay only. Progesterone concentrations were similarly reduced in ewes given a CIDR and fed lupins after mating compared with those given a CIDR and fed hay only *(*$P < 0.05$). Pregnancy rate and the number of foetuses per ewe pregnant were not significantly different between Groups 1, 2 and 3. Treatment with CIDRs significantly *(*$P < 0.05$) increased the pregnancy rate of ewes in Group 3, but not in Group 1 or 2.

*Keywords:* progesterone, lupins, embryo survival, breeding ewes.

## INTRODUCTION

Supplementary feeding of lupin grain at the time of joining has been shown to increase significantly the ovulation rate of Merino ewes, percentage of ewes lambing and percentage of ewes with twin lambs (Lightfoot and Marshall 1974, 1976). This increase in ovulation rate is not always followed by an increased number of lambs born (Croker *et al.* 1985). The reduction in the number of potential lambs from ewes eating lupin supplements most likely occurs due to increased embryo mortality (Brien *et al.* 1977). It is proposed that the feeding of lupins after ewes have mated causes a reduction in the concentration of peripheral plasma progesterone similar to that reported in overfed ewes (Parr *et al.* 1987). This causes embryonic mortality in ewes in which progesterone concentrations are below threshold levels at a critical time of early pregnancy. The aim of this study was to determine the effects of feeding lupins after joining on peripheral progesterone concentrations and embryo survival in Merino ewes.

## MATERIALS AND METHODS

### *Animals and breeding management*

Mature Merino ewes *(n* = 300) were grazed on sparse annual pasture at VIAS Werribee during the experimental period (March 1991). The ewes were maintained in store to forward store condition (condition score 2.5-3) with supplements of good quality meadow hay. Ewes were given an intravaginal CIDR device containing 300 mg progesterone (Eazi-breed CIDR G, Carter, Holt, Harvey Plastic Products, N.Z.) which remained in place for 12 days. Fertile Merino rams, fitted with harnesses and crayons (5 per 100 ewes) were placed with the ewes 2 days before CIDR withdrawal and mating occurred over a two day period, 48 to 72 h later.

### *Feeding treatments*

A supplement of lupin grain *(Lupinus angustifolius,* cv. Unicrop) was fed to ewes of Groups 1 and 2, for 9 days prior to the expected oestrus. The lupins were fed at a daily rate of 750 kg per head and replaced half of the daily hay supplement for these groups. Ewes in Group 1 continued to receive lupins for 14 days after mating and those in Groups 2 and 3 were fed a hay only supplement during this period.

*Experimental procedures*

On days 6 and 7 after mating (day of oestrus detection = day 0), all ewes were examined with an endoscope (Roberts 1968) and the ovulation rate (number of CL per ewe) was recorded. Half of the ewes in each group, chosen at random, were given a CIDR device on day 10. All ewes were blood sampled on day 12 by jugular venipuncture and plasma was stored at –200°C for later progesterone determination. On day 14, CIDRs were removed and all ewes were grazed as 1 flock. Harnessed vasectomized rams were used to detect returns to oestrus and pregnancy rates were confirmed using real-time ultrasonography at days 65-66 when foetal numbers were recorded.

*Progesterone assay*

Concentrations of plasma progesterone were determined using a direct assay kit (Farmos Diagnostica, Finland). The assay used a progesterone antiserum which had a negligible cross reactivity with other endogenous pregnen derivatives and related steroids. The binding of progesterone to serum proteins was inhibited by the inclusion of 17α-hydroxy-4-pregnen-20yn-(2,3d)-isooxazol in the tracer solution. Final separation of free and antibody-bound hormone was performed by precipitating the latter with a second antibody and polyethylene glycol. The assay used [$^{125}$I] progesterone and had a sensitivity of 0.5 nmol/L. Intra- and inter-assay coefficients of variation were 5.09% and 1.96% respectively. Although not previously validated for sheep, the correlation coefficient *(R* value), for a set of the same sheep samples, between the kit assay and an extraction assay used in our laboratory was 0.996 (Parr 1991).

## RESULTS

Changes in mean liveweight prior to mating and in the 14 day post-mating period (Table 1) indicated that all ewes experienced an increase in liveweight prior to mating and decreases in liveweight after mating, representing losses of 1.1, 2.7 and 7.7% of mating liveweights in Groups 1, 2 and 3 respectively. Lupin feeding prior to mating significantly *($P < 0.05$)* increased the ovulation rate of ewes when compared with feeding hay alone (Table 1). There was a significantly *($P < 0.05$)* higher proportion of ewes with multiple ovulations in combined groups fed lupins (Groups 1 and 2) than in the group fed hay alone (Group 3).

**Table 1. Mean (± s.e.) change in liveweight (LW, kg) and mean (± s.e.) ovulation rates (number of *corpora lutea*, CL, per ewe ovulating) of ewes fed lupins before and after mating (Group 1), ewes fed lupins before mating and hay after (Group 2) and ewes fed hay before and after mating (Group 3)**

| Group | LW change around mating | | Ovulation rate[A] |
|---|---|---|---|
| | Day -14 to -2 | Day -2 to 14 | (CL per ewe ovulating) |
| 1 (lupins/lupins) | 1.4 (± 0.20) | -0.6 (± 0.24) | 1.65a (± 0.06) |
| 2 (lupins/hay) | 1.9 (± 0.22) | -1.4 (± 0.19) | 1.52ab (± 0.05) |
| 3 (hay/hay) | 1.8 (± 0.23) | -3.9 (± 0.20) | 1.46b (± 0.08) |

[A]Means with different letters are significantly different ($P < 0.05$)

Within ovulation class, (1 or 2+ CL), mean peripheral plasma progesterone concentrations, measured on day 12, were significantly *($P < 0.05$)* reduced in ewes fed lupins after mating compared with those fed hay (Table 2). Although treatment with CIDRs increased mean progesterone concentrations, this effect of lupins was also evident in ewes given exogenous progesterone (Table 2). Pregnancy rates (ewes pregnant per ewes joined) and the number of foetuses per ewe pregnant were similar in Groups 1, 2 and 3. Treatment with exogenous progesterone significantly *($P < 0.05$)* increased pregnancy rate in Group 3 but had no effect on pregnancy rate or foetuses per ewe pregnant in any other group (Table 2). The difference in mean foetal number per ewe pregnant between ewes treated with a CIDR and control ewes in Group 3 was not significant *($P > 0.05$).*

## DISCUSSION

Supplementation with lupin grain during the 9 days before mating increased the ovulation rate of Merino ewes independently of changes in liveweight; this is in agreement with the results of Lightfoot and Marshall (1976). The continuation of feeding lupin supplements after mating resulted in a

**Table 2. Mean (± s.e.) concentrations of peripheral plasma progesterone (ng/mL), measured on day 12 after mating, percentage of ewes pregnant per ewes joined (EP/EJ) and mean (± s.e.) foetuses per ewe pregnant in ewes with or without a controlled internal drug release (CIDR) device from day 10-14 of pregnancy**

Within columns, means with different letters are significantly different (a, b $P < 0.05$; x, y $P < 0.001$)

| Group | CIDR | Plasma progesterone (ng/mL) 1CL | 2CL | EP/EJ (%) | Foetuses/EP |
|---|---|---|---|---|---|
| 1 (lupins/lupins) | + | 5.2x (± 0.20) | 5.5x (± 0.27) | 53ab | 1.41 (± 0.09) |
| | – | 3.6a (± 0.18) | 4.3a (± 0.25) | 55ab | 1.32 (± 0.09) |
| 2 (lupins/hay) | + | 6.9y (± 0.33) | 8.3y (± 0.31) | 58ab | 1.38 (± 0.08) |
| | – | 4.3b (± 0.25) | 5.0b (± 0.21) | 67a | 1.45 (± 0.08) |
| 3 (hay/hay) | + | 7.9y (± 0.54) | 8.3y (± 0.38) | 68a | 1.26 (± 0.09) |
| | | 4.7b (± 0.41) | 5.9b (± 0.49) | 42b | 1.46 (± 0.09) |

significant reduction in the peripheral plasma progesterone concentration, measured on day 12 after mating. It is suggested that this reduction is caused by a change in the rate of blood flow through the gut, to the liver, causing an increased rate of progesterone metabolic clearance without concomitant changes in the hormone's rate of secretion (Parr 1991). A similar reduction of peripheral progesterone in ewes fed lupins was reported by Brien et *al.* (198 1), but the mechanism at that time was unclear.

The pregnancy rates and number of foetuses per ewe pregnant were not affected by this reduction in plasma progesterone, indicating that embryonic mortality was not influenced by feeding lupins during the post-mating period. This is in contrast with the study of Brien *et al.* (1977) in which embryonic survival was reduced when ewes were fed lupin supplement for 12 days after mating. It is apparent that concentrations of peripheral plasma progesterone in the current study were not reduced sufficiently to endanger embryonic survival. Parr *et al.* (1987) found that concentrations of peripheral plasma progesterone of at least 2 ng/mL were necessary at day 12 for satisfactory conception. Since mean concentrations of progesterone at day 12 in this study were 3.6 ng/mL in ewes with single ovulations and fed lupins, it is concluded that few ewes would lose embryos due to a deficiency of progesterone. This is supported by the lack of response to progesterone supplement in these groups. The significant reduction in pregnancy rate in Group 3 ewes, mainly because of embryonic losses in single ovulating ewes, is paradoxical. This loss was prevented by treatment with exogenous progesterone, yet this group had the highest concentration of endogenous progesterone of the 3 groups.

In conclusion, it was found that feeding lupins prior to joining increased ovulation rates independently of changes in liveweight. The continuation of lupin feeding during the first 14 days of pregnancy caused a reduction of peripheral plasma progesterone concentrations measured on day 12, but this reduction did not affect embryonic survival.

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