Oestrus, ovarian activity and embryo survival in ewes grazing high and low oestrogenic red clover

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ABSTRACT

In two trials, ewes (n = 16 per group) that were potential recipients for embryo transfer grazed on a high oestrogenic red clover (Pawera), a newly developed low oestrogenic red clover (G27) and Ryegrass-White clover (Control) pastures. The ewes grazed the respective pastures during 12 days of intravaginal sponge insertion, and for a further 23 days after induced oestrus. The mean (± sd) interval from sponge removal to oestrus was shorter ($P<0.05$) in control (42.0 ± 12.1 h) than Pawera (58.5 ± 16.0 h) and G27 (52.6 ± 13.0 h) groups in trial 1 and comparable intervals in trial 2 were 42.3 ± 6.3 h, 46.3 ± 4.5 h and 51.3 ± 17.0 h respectively. In both the trials, number of ovular ewes and ovulation rate were lower ($P<0.05$) in Pawera ewes. The mean (± sd) ovulation rate in Pawera, G27 and control ewes in trial 1 was 0.63 ± 0.62, 1.63 ± 0.71 and 1.93 ± 1.00; it was 0.31 ± 0.71, 1.17 ± 0.93 and 1.53 ± 0.52 for the three groups in trial 2. Following the transfer into suitable recipients of two embryos per ewe post mortem examination at 35 days showed an implantation rate of 50%, 90% and 85% in Pawera, G27 and control groups in trial 1 and 50%, 50% and 69% in trial 2. It was concluded that G27, a low oestrogenic red clover is safer than Pawera red clover in terms of oestrous activity and ovulation rate when grazed around mating.

Keywords: Formononetin, phytoestrogen, red clover, ewe, embryo transfer, reproductive performance.

INTRODUCTION

Temporary infertility occurs in ewes grazing oestrogenically active clover prior to, and during the time of mating. The ewes exhibit an increase in return to service (Morley *et al.*, 1966; Kelly *et al.*, 1980; Shackell and Kelly, 1984), a reduced oestrous incidence (Lightfoot and Wroth, 1974; Kelly and Shackell, 1982), oestrus without ovulation (Ch'ang, 1958; Kelly *et al.*, 1980), decreased ovulation rate (Lightfoot and Wroth, 1974; Kelly *et al.*, 1980) and higher total losses of pregnancy on a ewe basis (Lightfoot and Wroth, 1974). It appears that the period of recovery from this temporary infertility may be related to the period of exposure to oestrogenic clover (Shackell and Kelly, 1984).

It is generally agreed that the substances responsible for the disorder are present mainly in the laminae of pasture legumes. The substances considered responsible for the oestrogenic activity are the isoflavones (Davies, 1987) among which formononetin plays the principal role (Cox and Braden, 1974).

Grasslands Pawera, like some other red clovers, has been shown to contain high levels of oestrogenic compounds. Pawera leaf may contain up to 1.4% of the isoflavone formononetin (Kelly *et al.*, 1979) and the reproductive performance of ewes is markedly impaired when pure swards of Pawera are grazed during joining (Kelly *et al.*, 1980). So it would be unwise to graze ewes for extended periods on pure Pawera, particularly at mating time (Hay *et al.*, 1978).

One method available to control the problem is to use red clover cultivars free of or low in formononetin. G27 red clover is a sixth generation selection within the cultivar Pawera, based on decreasing the formononetin levels in leaflets. Chemical assays have shown G27 red clover to have approximately 40% of the formononetin level of Pawera clover on a whole plant basis. This paper reports the results of two trials carried out to compare some aspects of reproductive performance in ewes grazing either Pawera red clover, G27 red clover, or ryegrass-white clover pastures. Embryo transfers were done to overcome possible fertilization failures in ewes grazing red clover and also to examine embryo survival on oestrogenic pastures.

MATERIALS AND METHODS

In two trials, six to seven years old, non pregnant Romney ewes (n = 16 per group per trial) grazed on Pawera red clover, G27 red clover or ryegrass-white clover (control) pastures. The ewes had a history of normal fertility and had not grazed on oestrogenic pasture before. Pawera and G27 were sown as pure red clover plots and at the time of grazing contained 70 to 90% red clover. Trial 1 (n = 48) was started on 2nd May 1991 and Trial 2 (n = 48) on 21st February 1992. In each trial ewes were split into two blocks for convenience and the ewes were synchronized two weeks apart. The ewes were grazed on the various pastures during 12 days of intravaginal sponge insertion and for a further 23 days after induced oestrus.

Intravaginal Progestagen sponges (containing 40 mg medroxy.progesterone acetate) were inserted and ewes were placed on treatment pastures. The sponges were removed on day 12. Each sheep received 300 IU PMSG (Folligon) at the

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time of sponge removal. Harnessed teaser rams were run with each group and marks were checked twice daily during the period 24-96 h after sponge removal. Ovulation rate was determined 5-6 days after heat by laparotomy or laparoscopy and ovular ewes received two good quality embryos from (Booroola x Romney) x Perendale ewes grazing ryegrass-white clover pasture (day 19-20 of the trial). The embryo recipient ewes continued grazing treatment pastures for another 18 days when the ewes from the red clover were moved to ryegrass-white clover (day 38). Recipient ewes were slaughtered 35 days after embryo transfer (day 55) to check embryo survival and pregnancy status.

Samples of red clover leaves and upper parts of stem were taken on three occasions during each trial and formononetin levels were determined using a fluorimeteric assay (Gosden and Jones, 1978).

STATISTICAL ANALYSES

Data of mean interval from sponge removal to oestrus was analyzed by Analysis of Variance procedure. Number of marked ewes, ovular ewes, and ovulations per ewe were compared by Chi square method.

RESULTS

Mean formononetin levels for the treatment pastures are given in Table 1. Pawera red clover had formononetin levels more than twice that of G27.

TABLE 1: Formononetin levels (% of herbage dry matter) in treatment pastures

Herbage	Trial 1 mean±sd	Trial 2 mean±sd
Pawera	1.31 ± 0.14	1.09 ± 0.13
G27	0.59 ± 0.11	0.34 ± 0.08
Control	Undetectable	

Onset of oestrus

The occurrence of oestrus and ovarian activity in the three treatment groups are shown in Table 2. The percentage of marked ewes was lowest in the Pawera groups but the differences were not significant. In trial 1 mean interval from sponge removal to oestrus was similar in Pawera and G27 groups but it was shorter ($P<0.05$) in the control group. In trial 2 the interval was again shortest for the control group, but there was no significant difference between the three groups.

Ovulation rate

Percentage of ovular ewes and ovulation rate were similar for G27 and control ewes but were significantly lower ($P<0.05$) in Pawera ewes. Incidence of oestrus without ovulation was quite high in ewes grazing Pawera red clover (36% and 75% in trial 1 and 2 respectively) but it was only 6% and 25 % for two trials on G27 red clover. The non ovulating ewes had some follicles or they had flat ovaries as examined on day 5 after oestrus. In the second trial two unmarked Pawera ewes had mid cycle corpora lutea on the ovaries and one anovular G27 ewe had a luteinized follicle.

All the Pawera group ewes had markedly enlarged udders and their uteri were tense and oedematous.

Embryo survival

Embryo survival results for the two trials are presented in Table 3.

TABLE 3: Results of embryo transplantation (2 embryos/ewe)

Group	No. of ewes implanted	Embryos surviving No.	Embryos surviving %	Pregnancy status No.	Pregnancy status %
Trial 1					
Pawera	5	5	50	3	60
G27	10	18	90	10	100
Control	10	17	85	10	100
Trial 2					
Pawera	2	2	50	1	50
G27	9	9	50	5	56
Control	13	18	69	10	77

Owing to a lower number of suitable embryos in Trial 1, only 25 ewes out of 38 that ovulated were transplanted with embryos.

TABLE 2: Oestrus and ovarian activity in ewes

Group	Number in treatment	Number marked(%)	Hours to oestrus mean±sd	Ovular ewes %	Number of ewes with 0 ovulations	1 ovulations	≥2 ovulations	Ovulation rate mean±sd
Trial 1								
Pawera	16	14 (88)	58.5 ± 16.0[a]	56[a]	7	8	1	0.63 ± 0.62[a]
G27	16	16 (100)	52.6 ± 13.0[a]	94[b]	1	5	10	1.63 ± 0.71[b]
Control	14*	13 (93)	42.0 ± 12.1[b]	100[b]	0	4	10	1.93 ± 1.00[b]
Trial 2								
Pawera	16	12 (75)	46.3 ± 4.5	19[c]	13	1	2	0.31 ± 0.71[c]
G27	12**	12 (100)	51.3 ± 17.0	75[d]	3	5	4	1.17 ± 0.93[d]
Control	13***	13 (100)	42.3 ± 6.3	100[d]	0	7	6	1.53 ± 0.52[d]

Groups with different superscripts in the same column and in the same trial are different at the $P<0.05$ level.

* Two ewes were lost

** Four ewes lost sponges

*** Three ewes lost sponges

Embryo survival and pregnancy rate (35 days after embryo transfer) were similar for G27 and control groups in trial 1. In trial 2 survival rate was highest in the control group. No statistical analysis was performed owing to the low number of embryos transferred especially among the Pawera ewes.

DISCUSSION

G27 red clover which has formononetin levels less than half of Pawera red clover is safer in terms of number of ovular ewes and ovulation rate in the ewes grazed on this pasture around oestrus.

Two weeks of grazing ewes during oestrous synchronization on G27 resulted in an ovulation rate comparable to that on ryegrass-white clover pasture. There were a few marked ewes (4 of 28 for both trials) that failed to ovulate on G27 red clover but the number of such ewes was significantly higher on Pawera red clover (14 of 26 marked ewes). Mean ovulation rate was also significantly lower ($P<0.05$) on Pawera red clover as compared to G27 and control pastures. The results for Pawera were consistent with some earlier studies. Lightfoot and Wroth (1974) recorded a lower ovulation rate for the oestrogenic group (1.13 vs 1.51). Kelly *et al.*, (1980) observed a mean ovulation rate of 0.79 in Romney ewes grazing Pawera red clover vs 1.55 on control pasture for 8 days before and during the first cycle of mating. Out of 24 anovular ewes in their study, 69% had been marked by the ram. Ch'ang (1958) also reported that some Romney ewe lambs showed oestrus without ovulation after grazing on a red clover pasture.

Interval from sponge removal to heat was lowest in control group ewes in trial 1 ($P<0.05$) but the differences were nonsignificant between the three groups in the second trial. As large errors in the time of heat existed, no conclusion was made in regard to possible treatment effect.

Embryo survival rate after transfer of good quality fertilized eggs to the ewes (2 eggs per ewe) was similar on G27 and control pastures as observed at slaughter 35 days post transfer. As too few Pawera ewes received embryos, the effect on embryo survival could not be determined. According to Kelly *et al.*, (1980) embryonic mortality, as measured by the difference between the number of ovulations and the number of lambs born to the cycle in which the ewe was last marked was not significantly different between the Pawera and white clover-grass groups. In the present study the method used to check embryo survival was not successful for Pawera due to the low number of ovular ewes. Variability in superovulatory response also played a role as insufficient fertilized eggs were available in the first trial.

The results of this study suggest that G27, a newly developed low oestrogenic red clover is safer than Pawera red clover when grazed for two weeks before oestrus. It should be mentioned here that another selection has been made (from within G27) to further lower the formononetin levels since this experiment was carried out.

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