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The effect of ram replacement and sex ratio on the sexual response of anoestrous ewes

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Abstract

It is accepted that an important source of variation in the response of anoestrous ewes, to the introduction of rams, is the intensity of male stimulation. The aim of this study was to investigate strategies capable of increasing the impact and transmission of the ram stimuli. In Experiment 1, two groups of seven ewes (Bluefaced Leicester♂ × Swaledale♀) were individually penned with one ram and for the next 6 h the rams either remained in the pen or were replaced hourly. Blood samples revealed no difference in the pattern of plasma LH secretion. In Experiment 2, three groups of 16 ewes were either introduced to one ram, individually (H) or in groups of 8 (L), or remained isolated. Ram introduction increased the plasma LH pulsatility ($P < 0.001$). H ewes displayed more (nine versus six) male-induced LH pulses (pulses occurring within the first 45 min) and more pulses per 8 h intervals than the L group of ewes (1.9 ± 0.3 versus 1.3 ± 0.3), but these differences were not significant. It was concluded that (i) frequent replacement of rams within a few hours following ram introduction to ewes does not further improve the response of ewes, especially if the ram:ewe ratio is high; (ii) the characterization of the plasma LH secretion parameters during a period of 6–8 h does not seem to be an effective method to detect small differences in the intensity of stimulation received by the ewes when exposed to rams; (iii) North Country Mule ewes (Bluefaced Leicester♂ × Swaledale♀) in the UK respond to the presence of rams in spring (late oestrous/early anoestrous season) with an elevation in plasma LH secretion.

Keywords: Sheep; Reproduction; Seasonality; Ram effect; Anoestrus

1. Introduction

It is well established that the stimuli involved in the ram effect are of pheromonal and behavioural origin, and attempts have been made to increase the intensity of these stimuli—aiming at increasing the response

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of ewes to the introduction of the rams (Pearce and Oldham, 1988; Rosa et al., 2000a,b; Rosa and Bryant, 2002). Anoestrous ewes generally need to be primed by a period of isolation from rams before responding to the ram effect (Oldham, 1980; Knight, 1983; Martin et al., 1986) and most ewes which remain anovular after experiencing a period of close contact with male goats (McMillan, 1987) or rams (Pearce and Oldham, 1988)—subsequently ovulating in response to the introduction of new rams. This suggests that ewes accustom to particular rams, still maintain a sensitivity to the stimulatory effect of other males. On the other hand, the ewe-seeking activity and the courtship behaviour of the rams decrease markedly with time after introduction (Rosa, 1998). Thus, the behavioural component of the ram stimulus may be much more intense in the period immediately after joining. From the preceding considerations, it can be speculated that the frequently alternating of male partners will increase the sexual stimulation in ewes and consequently improve their response to the 'ram effect'.

The intensity of sexual stimulation experienced by the ewes may result from the individual pheromone production, sexual behaviour displayed by the rams and from the number of rams present (i.e., the ram:ewe ratio) (Signoret et al. (1982)). As far as the ram to ewe ratio is concerned, researchers have not devoted enough attention to this phenomenon and the effect of this factor is probably underestimated.

The literature reports a wide range of ram to ewe ratios under different production systems and all are assumed to be sufficient to stimulate females (1:1, O'Callaghan et al., 1994; 1:25, Thompson et al., 1990; 1:75, Burfening et al., 1989; 1:100, Signoret et al., 1982). However, Signoret et al. (1982) recorded a significant increase in the number of ewes ovulating when this ratio was changed from 1:100 to 1:20. Rekik (1988) also recorded an unexpected oestrous response of 100% with a ram to ewe ratio of 1:4. In a previous study on the same flock only 10 of the 32 ewes ovulated when using a ratio of 1:10. However, Rekik (1988) had pre-treated the rams with testosterone which could have increased their pheromone production (Fulkerson et al., 1981; Knight, 1983; Martin et al., 1986; Haynes and Haresign, 1987).

The objective of this study was to determine the rate of sexual response in seasonal anoestrous ewes to ram introduction, with an increased frequency in the alternation of males and an increased ram to ewe ratio.

2. Material and methods

2.1. Animals and treatments

The experiments took place at the Reading University (latitude 51°27′N). The mean (±S.D.) live weight of the ewes was 80.8 ± 8.2 kg and 82.9 ± 10.1 kg for the Northcountry Mules and Welsh Mules, respectively. The ewes lambed in February (winter) and were weaned at the beginning of April (spring).

All ewes were prevented from visual, sound and odor contact of rams for several months being isolated at a distance of at least 1 km from the males. All rams were adult (aged 4 years) and sexually experienced.

2.1.1. Experiment 1

Fourteen Northcountry Mule (Bluefaced Leicester♂ × Swaledale♀) ewes and 14 Texel rams were randomly allocated according to live weight to the following two treatments:

Treatment 1: Seven ewes were individually penned and one ram was introduced to each pen for 6 h (control group; C). Treatment 2: Seven ewes were individually penned and one ram was introduced to each pen. The rams were then replaced hourly by a new fresh ram for a period of 6 h (Group R). Fresh in this context implied a ram had been isolated from ewes for at least an hour. The rams were used in rotation so that no ewe interacted the same ram more than once during the experimental period.

The rams were introduced in April (early spring) at 8:00, with an interval of 3 days separating the treatments. The experiment took place in closed barns.

2.1.2. Experiment 2

In July (early summer), 48 ewes of which 24 were Northcountry Mule (Bluefaced Leicester♂ × Swaledale♀) and 24 were Welsh Mule (Bluefaced Leicester♂ × Beulah♀) and 14 Texel rams were randomly allocated, according to breed and live weight, to the following three treatment groups:

Treatment 1: Ewes ($n = 16$) were kept in isolation from rams throughout the experimental period (control group; C). Treatment 2: Ewes ($n = 16$) were individu-

ally penned and one ram was introduced to each pen (Group H). Treatment 3: Ewes ($n = 16$) were housed in groups of 8 in a large open barn and one ram was introduced per group (Group L).

Immediately after the introduction of the rams, blood was sampled at 15 min intervals from ewes for a period of 6 h (Experiment 1) and 8 h (Experiment 2). In Experiment 2, each group of 16 ewes was divided into two sub-groups of 8 (4 Northcountry Mule and 4 Welsh Mule) and in each subgroup blood was sampled on alternate days (according to the following sequence: first subgroup C; first subgroup L; first subgroup H; second subgroup C; second subgroup L; second subgroup H). The rams were allowed to move freely inside the barn and had limited physical contact with the ewes.

2.2. Evaluation of the plasma LH response in the ewe

The primary endocrine response of the ram effect is an increase in the pulsatility and tonic secretion of LH (Knight, 1983; Martin et al., 1986; Al-Mauly et al., 1991), which will culminate in most cases in a pre-ovulatory plasma LH surge and ovulation. The criteria chosen to assess the sexual response was the plasma LH profile in the ewes during the first 6–8 h following ram introduction. For this purpose blood samples were collected at 15 min intervals, the first sample being taken immediately after the introduction of the ram.

The parameters used to characterize the pattern of plasma LH secretion were the pulse frequency (pulses per hour), the basal level (ng/ml) and the pulse amplitude (ng/ml) (Goodman and Karsch, 1980; Atkinson and Williamson, 1985; Cohen-Tannoudji et al., 1986). The criteria used to identify an LH pulse were: (i) a rapid rise from the basal plasma LH level to a peak value which occurred within 30 min (two samples), followed by an exponential-type decline to baseline concentrations, (ii) the plasma LH level at the peak had to exceed the 95% confidence limits of the basal level and (iii) the amplitude had to be greater than the sensitivity of the plasma LH assay. The amplitude of each plasma LH pulse was defined as the plasma LH concentration at the peak, minus that at the preceding lowest value. The basal plasma level was determined as the mean of all values not considered to be part of a pulse.

The response of the ewes to ram introduction was also assessed by the occurrence of male induced plasma LH pulses. This, however, could not be used to compare treatments in Experiment 1 (male induced pulse was considered a pulse that occurred before the fifth sample, i.e., within 45 min following the onset of stimulation) (Cohen-Tannoudji et al., 1986).

2.3. Blood sampling procedure

In order to make frequent blood sampling possible, a catheter (silastic tubing with 38 cm length, 1.00 mm inside diameter and 1.60 mm outside diameter) was inserted to a depth of 10 cm in the jugular vein of the ewes under local anesthesia, the day before the onset of the experiment. Five millilitres blood was collected in heparinized syringes and immediately transferred to centrifuge tubes cooled on ice. After centrifugation at 4 °C and 2000 × *g* for 15 min, the plasma was decanted and stored at −20 °C until assayed for plasma LH.

2.4. LH hormone assay

Levels of ovine plasma LH (OLH) were measured using a specific double-antibody radioimmunoassay (Walton et al., 1977). The antiserum used (RO7/5) was raised in rabbits against NIH-LH-S16 and was used at a final dilution of 1:14,000, which is required to bind 30% of the labeled hormone. Sheep LH was radiolabelled with [^{125}I] using chloramine T (Greenwood et al., 1963). Standard ovine LH (NIH-LH-S16) was used to make the following range of standard dilutions: 0.2, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5 and 25.0 ng/ml. One hundred microlitres of each of these standard concentrations were added to four disposable plastic tubes. Four tubes to determine the non-specific binding (NSB), received 150 μl of radioimmunoassay diluent (RIAD), another set of four tubes for the estimation of radioactivity binding in the absence of unlabelled hormone (B MAX) received 100 μl of RIAD. Plasma samples (100 μl) was added to two tubes. Four replicates of the control plasma sample (3.56 ± 0.089 ng/ml; S.E.M., for Experiment 1 and 0.468 ± 0.0072 ng/ml; S.E.M., for Experiment 2) were included at the beginning and the end of the assay, in order to establish the precision of the assay. Fifty microlitres of antiserum were added to all tubes with the exception of the NSB. The tubes were then vortexed and incubated overnight at room temperature. Fifty microlitres [^{125}I] OLH (8000–10,000 cpm) was then added to all tubes,

plus a new set of four, to determine the total cpm (TC). After vortexing, the tubes were incubated for a further 24 h at room temperature and 200 μl of the second antibody was added to all tubes except the TC. The tubes were vortexed and left at room temperature for 45 min. All tubes were then centrifuged at $1600 \times g$ for 30 min at 4 °C to separate the antibody-bound and free-labeled hormone. The supernatant was aspirated and radioactivity in the precipitates counted for 60 s in a LKB gamma counter. The samples were analysed in 12 assays. The mean non-specific binding was 2.6% ± 0.97 (S.E.M.) and the cross reaction of the antiserum with ovine FSH was 0.3% and with other pituitary hormones was <0.01%, as reported by Walton et al. (1977). Regarding the sensitivity, the concentrations of OLH standard required to inhibit the binding of the [^{125}I] OLH to the antiserum RO7/5 by 20, 50 and 80% were 0.329 ng/ml (±0.014), 0.888 ng/ml (±0.025) and 2.483 ng/ml (±0.04), respectively. The mean intra-assay and inter-assay coefficient of variation was 7.4 and 8.5%, respectively.

2.5. Statistical analysis

Differences between the plasma LH basal levels and LH pulse amplitudes were compared by Student's *t*-test (Experiment 1) and ANOVA (Experiment 2). Data of pulse frequency and male induced pulses were arranged in frequency distribution tables and analysed by the Fisher exact probability test (SAS, 1989).

3. Results

3.1. Experiment 1

The plasma LH concentrations increased from approximately 1 ng/ml to a maximum of 11 ng/ml within 15 min. The mean pulse frequency was the same in both treatments (0.33 pulses/h) (Table 1). The number of pulses recorded in individual ewes ranged from 1 to 4 and from 1 to 3 in Treatment C and R, respectively, with no significant difference being recorded when the ewes were distributed in the categories of the number of pulses displayed. In the two treatments, 10 ewes (71.4%) displayed a male induced pulse.

3.2. Experiment 2

Fifteen out of 32 ewes (47%) of Treatments L and H showed a male induced LH pulse. These induced pulses were more frequent in Treatment H (nine versus six, 50% more), but this difference was not significant (Table 2). The basal level and the amplitude of plasma LH pulses were very similar in the three treatments. The small differences recorded never reached a significant level and the data did not suggest any tendency for these parameters to be affected by the treatments (Table 2). In contrast, the pulse frequency was clearly influenced by the presence of the rams. Despite the fact that the data did not follow a normal distribution (which consequently prevented the means in Table 2 to be compared statistically), the arrangement of data in a frequency distribution table (Table 3) demonstrated that ewes in Treatments L and H displayed significantly more LH pulses than the control ewes (L versus control; $P < 0.05$ and H versus control; $P < 0.001$). The H treated ewes displayed on average almost 50% more pulses than the L treated ewes (the proportion of ewes displaying two or more pulses were 60.0 and 43.8% in treatments H and L, respectively), but the differences were not significant.

4. Discussion

It could be hypothesised (Experiment 1) that the successive contact with alternative rams have an additional

Table 1
Effect of hourly replacement of rams on plasma LH secretion (mean ± S.E.) in anoestrous ewes during the first 6 h of contact with rams

Treatment	Pulse frequency			Basal Level (ng/ml)	Amplitude (ng/ml)	No of male induced pulses
	Pulses per 8 h	Pulses per hour	Hours between pulses			
C (rams not replaced) ($n = 7$)	2 ± 0.37	0.33	3	1.70 ± 0.11	5.04 ± 0.78	6
R (rams replaced) ($n = 7$)	2 ± 0.22	0.33	3	1.44 ± 0.11	3.58 ± 0.40	4

Differences in basal level and amplitude of pulses are not statistically significant.

Table 2
Effect of different degrees of ram stimulation on plasma LH secretion (mean ± S.E.) in anoestrous ewes during the first 8 h of contact with rams

Group	Pulse frequency			Basal Level (ng/ml)	Amplitude (ng/ml)	No. of male induced pulses
	Pulses per 8 h	Pulses per hour	Hours between pulses			
Control ($n = 16$)	0.81 ± 0.19	0.10	10	0.35 ± 0.02	1.68 ± 0.23	–
L ($n = 16$)	1.31 ± 0.25	0.16	6.3	0.38 ± 0.03	1.80 ± 0.31	6
H ($n = 15$)	1.93 ± 0.28	0.24	4.2	0.38 ± 0.04	1.36 ± 0.21	9

L = Lower stimulation; H = Higher stimulation. Differences in basal level, amplitude of pulses and number of ewes showing one induced pulse are not statistically significant.

stimulatory effect in ewes, leading to increased sexual response to the presence of rams. Not only because of alternating rams itself, but also because of the willingness of the rams to seek and court the ewes. The results clearly showed that the plasma LH pulsatility of ewes does not increase further during the first few hours of ram contact as a result of such stimulation. Pearce and Oldham (1988) reported the introduction of new rams to generate additional stimulation which leads to a greater proportion of ewes responding. In this study, the response was assessed by ovulation, which occurs 2–3 days after ram introduction and the new rams were introduced several weeks after the original introduction of rams, which may represent enough time for the ewes to have become used to the original rams. On the other hand, the control ewes in this experiment were penned individually with one ram which may have represented a strong stimulation and contributed to a lack in difference of response between treatments. It would be interesting to test a similar protocol (i.e., frequent replacement of rams in the first few hours of ram introduction) under field conditions, using a control group with a lower ram to ewe ratio and having the proportion of ewes ovulating as the response criterion.

The hypothesis that a higher ram to ewe ratio can positively affect the efficacy of the ram effect was also not confirmed by the results in Experiment 2. However, although not significant, superiority in Treatment H existed in terms of mean pulse frequency (48%) and the number of male induced pulses (50%). Considering this, together with the large variation in response within each treatment, with the relatively small number of animals in each treatment for the variability observed (and the already high degree of stimulation received in the control group [ram:ewe ratio = 1:8]), it would seem reasonable not to discredit the hypothesis as the results are not incompatible. Considering the strong viability of the hypothesis, it seems that further investigations, carried out under field conditions, with less variable reproductive parameters of response (e.g., occurrence of ovulation) and involving a larger number of animals, would be very justifiable.

An interesting suggestion of this study was the ability of the ewes to respond to rams in spring. In Experiment 1, 10 out of 14 ewes displayed a male induced pulse and the plasma LH secretion pattern of 11 ewes was consistent with a response to ram introduction. Not only did the plasma LH concentration increase from a basal level of <1 ng/ml to 2–22 ng/ml (Martin et al., 1980), but also, 11 ewes displayed two or more pulses during the 6 h observation period. In Experiment 2, 15 of the 16 ewes kept isolated from rams displayed no more than one pulse during the 8 h blood sampling period. Additionally, many reports have indicated that ewes kept isolated from rams tend not to display more than one pulse per 8–12 h period (Martin et al., 1980; Karsch, 1984; Martin, 1984).

The control group in Experiment 1 and Treatment L of Experiment 2 were tested under similar conditions. The rams, pens and experimental procedures were the same and the only major difference was the season of ram introduction (spring and summer). Therefore, any significant difference recorded between these treatments can most probably be attributed to this factor.

Table 3
Distribution of the ewes according to the number of LH pulses displayed during the 8 h experimental period in the different treatments

Group (no. of ewes)	Pulses per 8 h		
	0–1	≥2	% ewes ≥ 2
Control ($n = 16$)	15	1	6.3
L ($n = 16$)	9	7	43.8
H ($n = 15$)	6	9	60.0

Control vs. L; $P < 0.05$ and control vs. H; $P < 0.001$.

The comparison between the two treatments shows that all the parameters in plasma LH secretion were higher in Experiment 1, e.g., pulses per hour (0.33 versus 0.24), basal level (1.70 ng/ml versus 0.38 ng/ml), amplitude of pulses (5.04 ng/ml versus 1.80 ng/ml), proportion of ewes displaying a male induced pulse (86% (6 out of 7) versus 38% (6 out of 16)). Significant differences in plasma LH basal levels, amplitude of the pulses and the proportion of ewes displaying a male induced pulse were recorded. These results suggest that the ewes are more sensitive to the ram effect in early spring than in early summer. Literature does not quote studies of the ram effect using Mule ewes (or breeds with a similar seasonality), in which rams were introduced in early spring (approximately the onset of the anoestrous season). In July (early summer), 6–100% of these ewes were found to ovulate in response to ram introduction (Rekik, 1988). Mule ewes in the UK are not expected to start ovulating naturally before late August/early September (late summer), while in mid-April (early spring), 20–25% are expected to still be cyclic (Mitchell et al., 1997). Considering that a positive correlation exists between the proportion of ewes that ovulate spontaneously in the flock ('depth' of anoestrus) and the proportion of anoestrous ewes that respond to ram introduction (Martin et al., 1986), at the end of the day it is not surprising that the data suggest a greater sensitivity in ewes to rams in early spring. The intriguing question of this study is whether the changes in plasma LH secretion observed in early spring are sufficient to promote a subsequent sustained increase in plasma LH, necessary for ovulation to occur. At this time, the animals are subject to an increasing sensitivity to the negative feedback action of oestradiol. This is of interest in the sense that the ram effect has not been used in the UK at this time of the year, and the possibility that the ewes could respond to rams in early anoestrus (despite the likely requirement of high ram stimulation) is a situation with big commercial implications.

Ewes, in this study, were challenged by a very high ram stimulation, not verified under farm conditions, but still many ewes failed to respond. What this work seems to suggest is that some ewes, probably those in 'slight' anoestrus, according to the definition of Oussaid et al. (1993), can respond to rams at any time during the anoestrous season, provided that ewes receive adequate stimulation. Other ewes, however, fall in a 'deep' anoestrus and become insensitive to rams, whatever the intensity of stimulation received.

In conclusion, it can be said that the results of this study suggest that: (i) the frequent replacement of rams within a few hours following ram introduction to ewes does not further improve the response of ewes to the ram, especially if the ram to ewe ratio is high; (ii) the characterization of LH secretion parameters during a period of 6–8 h is not an effective method to detect the effects of small differences in the intensity of stimulation received by the ewes when exposed to rams; (iii) Mule ewes in the UK can respond to rams in spring (late oestrous/early anoestrous season) by an elevation in the plasma LH secretion.

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