

STIMULATION OF OVULATION IN SEASONALLY OR LACTATIONALLY ANOVULAR EWES BY RAMS

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If preconditioned by a period of isolation from rams during the non-breeding season, ewes of many breeds respond to the reintroduction of rams (teasing) by displaying a relatively synchronised oestrus, approximately one oestrous cycle later (Merino: Schinckel 1954a; Awassi: Eyal 1958; Romney: Edgar and Bilkey 1963; Merinos d'Arles: Prud'hon *et al.* 1966). The oestrous activity of the flock is spread over approximately 10 days but characteristically with two peaks: the first around day 18, and the second around day 24 after teasing. In successfully teased ewes the first oestrus is preceded by a silent ovulation (ovulation without oestrus) within six days of the introduction of rams (Schinckel 1954b). Progesterone priming is necessary if behavioural oestrus is to accompany the ram-induced ovulation (Hunter *et al.* 1971).

The proportion of ewes which ovulate after teasing varies greatly. Some breeds, for example the Romney, will respond to teasing only during a very limited period just before the start of the spontaneous breeding season (Edgar and Bilkey 1963), while Merinos appear able to respond to teasing at almost any season of the year if they are anoestrous. The response in Merinos apparently varies according to the stage of the non-breeding season (Lishman 1969), strain of ewe (Watson 1962; Lishman and Hunter 1967), the period of isolation (Lishman and Hunter 1967) and the breeding rhythm (time of mating, lambing and weaning) of the flocks studied (Lyle and Hunter 1965).

Lack of response in some experiments may have been associated with inadequate isolation of the ewes from rams before teasing. When isolation from rams is required, yet the only variable measured (oestrus) involves the use of rams, it is not possible to define the reproductive state of experimental ewes before and during teasing (Schinckel 1954b). Furthermore, the times of entry to and exit from anoestrus, and the "depth" of anoestrus, vary markedly with strain of ewe (Watson 1962) and past nutritional or geographical factors (Hunter 1962; Smith 1965). Flocks in which most ewes are cycling spontaneously cannot be expected to respond to teasing regardless of the period of isolation, degree of isolation or time of the year. To study teasing further, a variable was required which could be successfully measured both before and after teasing treatments, yet was independent of rams. The development of laparoscopy (Oldham *et al.* 1976a) and rapid assays for blood progesterone (Thimonier 1978), enabled direct assessment of the cyclic state of the ovaries of ewes during the period of isolation before teasing. Neither method stimulates ovulation in seasonally anovular ewes, and the response of such ewes to rams is highly repeatable (Oldham *et al.* 1976b). In this system, successfully teased ewes are those seasonally anovular ewes which ovulate within 3-4 days of their reintroduction to rams and control ewes are either continuously associated with, or isolated from, rams.

RECENT ADVANCES USING THIS APPROACH

The minimum period of isolation from rams required by ewes before teasing will induce ovulation has yet to be determined, but Oldham (1980) has shown that 34 days is certainly sufficient, and that possibly a period as short as 17 days is sufficient for seasonally anovular Merino ewes during October in Western Australia. Similarly, the response of seasonally anovular Ile-de-France ewes (Nouzilly-France) isolated from rams for 21 days before teasing in June was the

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same as that of flockmates isolated for more than four months.

Rams do not need to be in physical or visual contact with the ewe to induce ovulation (Watson and Radford 1960) and ewes with an impaired sense of smell do not exhibit oestrus after being teased by rams (Morgan *et al.* 1972). These latter workers suggested that pheromones, produced by the ram, will stimulate ewes to ovulate. Testosterone-treated wethers will stimulate oestrus in anovular ewes while untreated wethers have no effect (Fulkerson *et al.* 1979). This suggests that androgens may control the pheromone production. Recent investigations into the source of the pheromone and possible factors affecting it, are reported in the second paper by T.W. Knight and P.R. Lynch from New Zealand.

Seasonally anovular and lactationally anovular ewes ovulate 30-72h after the start of teasing and the ovulation is preceded by a surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) commencing on average 27, 35, 27 and 20 h after teasing in Merino, Romney, Ile-de-France and Préalpes du Sud ewes respectively (Oldham *et al.* 1978; Knight *et al.* 1978; Cognie *et al.* 1978; Poindron *et al.* 1980). The preovulatory surge of LH is preceded by a dramatic increase in the frequency of pulses of LH within 10 minutes of introducing rams (Martin *et al.* 1980; Poindron *et al.* 1980). Due to the speed with which the LH surge is induced (Oldham *et al.* 1978), and the apparent lack of a preovulatory rise in levels of oestradiol-17 β in the peripheral plasma of some successfully teased ewes (Knight *et al.* 1978), it was hypothesised that there may be "reflex" ovulation in the ewe. However, better understanding of the exact mechanism awaits detailed study of hormones other than LH between teasing and ovulation. The most recent results in this area are presented in the third paper by G.B. Martin and colleagues.

The explanation for the biphasic spread of the first oestrus after teasing lies in the variable life span of the ram-induced corpus luteum (CL). Premature regression of 50-60% of these CL, followed by a second "silent" ovulation and a CL which persists for a normal life span, causes the second peak of oestrus around day 24 (Oldham and Martin 1978; Knight *et al.* 1980). Priming with progesterone ensures that all ram-induced CL persist for a normal life span (Oldham *et al.* 1980). In addition the ovulation rate of ewes ovulating at teasing is significantly increased above that of spontaneously ovulating flockmates, or their own next ovulation. Evidence for this and its possible application are explored further by Yves Cognie and co-workers in the fourth paper. D.G. Corke has used teasing to synchronize oestrus for large AI programmes, and his results are presented in the fifth paper.

In order to maximise productivity, particularly in Western Australia where many flocks are mated during anoestrus and the conception rate is only 50-60% (Lindsay *et al.* 1975a), it is essential that ewes which fail to conceive at the first oestrus return to the ram for a second or third chance. Ewes which are teased, fail to conceive, then become anoestrous rather than return to the ram, may represent a significant source of reproductive wastage. This possibility is explored in the final paper.

THE PHEROMONE FROM RAMS THAT STIMULATES OVULATION IN THE EWE

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The pheromone which induces ovulation in seasonally anovular ewes is

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apparently present in the skin and wool of rams, but not in the urine (Knight and Lynch 1980). In this paper we describe recent progress in the isolation, purification and characterization of the pheromone.

EXPERIMENTAL

In all experiments mature Romney ewes underwent laparoscopy on Day 1 and the anovular ewes were selected. Treatments were applied twice daily on days 1 and 2 and the proportions of ewes ovulating were identified on Day 5 by laparoscopy*. Treatment groups were separated by at least 100 m. There were 20 ewes per treatment in the four experiments. Comparisons between treatments were tested for statistical significance by Chi-square analysis. .

Experiment 1 Wax, collected from the flanks and around the eyes of Dorset rams, and mixed with twice the volume of petroleum jelly, was rubbed on the muzzles of T 1 ewes. T 2 ewes had petroleum jelly rubbed on their muzzles while T 3 ewes were kept isolated and T 4 ewes were teased with three Dorset rams for four days.

Experiment 2 T 5 ewes had wool from Dorset rams rubbed on their muzzles. The crude liquor obtained from washing Dorset rams' wool in water or in petroleum spirits was painted on to the muzzles of the T 6 ewes and T 7 ewes respectively. The T 8 ewes had petroleum jelly, which had been smeared on to the backs of Dorset rams, rubbed on their muzzles. Cotton wool soaked in the petroleum spirit liquor and with the petroleum spirits evaporated off, was rubbed on to the muzzles of the T 9 ewes. The T 10 ewes remained isolated.

Experiment 3 T 11 ewes had the crude liquor from washing wethers' wool in petroleum spirits painted on their muzzles. The T 12 ewes were isolated.

Experiment 4 Wethers and ewes were injected three times at seven day intervals with 105 mg testosterone propionate ("Tesgro", Merck, Sharp & Dohme). T 13 ewes had the wool from these wethers rubbed on their muzzles, while T 14, T 15 and T 16 ewes were teased for four days with four wethers or four ewes treated with testosterone, or with four Dorset rams respectively. T 17 ewes remained isolated.

RESULTS AND DISCUSSION

In Experiment 1 more ewes treated with wax ($P < 0.05$) or joined with rams ($P < 0.05$) ovulated than did isolated ewes or the ewes treated with uncontaminated petroleum jelly (Table 1).

In Experiment 2 the groups treated with rams' wool ($P < 0.01$), the aqueous liquor ($P < 0.01$), the petroleum spirit liquor ($P < 0.01$) or with petroleum jelly that had been smeared on the ram's back ($P < 0.05$), all had significantly more ewes ovulating than the isolated ewes (Table 1). The cotton wool with the residues from the petroleum spirit liquor failed to stimulate the ewes as did the petroleum spirit liquor from wethers' wool in Experiment 3.

These results confirm previous work (Knight and Lynch 1980) 'which indicated that rams produced pheromones which stimulate anovular ewes to ovulate. They dispel any suggestion that the effects were caused by stress during treatment or the solvents or vehicle used for applying the treatment. The results also indicate that the effects of the pheromones were not masked by any of the solvents.

The presence of the pheromones in the wool, wax from the flanks and around the eyes of the rams and in the petroleum jelly smeared on to the ram's back,

TABLE 1 Percentage of ewes ovulating in the treatments in the 4 experiments

Experiment 1	T 1	T 2	T 3	T 4		
% ewes ovulating	37	0	0	15		
Experiment 2	T 5	T 6	T 7	T 8	T 9	T 10
% ewes ovulating	63	55	70	45	5	10
Experiment 3	T 11	T 12				
% ewes ovulating	25	25				
Experiment 4	T 13	T 14	T 15	T 16	T 17	
% ewes ovulating	5	0	10	55	0	

would suggest that the pheromones are produced from glands over most of the ram's body. Ram urine induced ovulation in a small proportion of ewes (Knight and Lynch 1980) but this was probably due to contamination with pheromones from the wool. Since the pheromones were present in both the water and petroleum spirit washings of the rams' wool, it appears that they are produced by the sudoriferous glands and are secreted with the suint. Suint is soluble in both water and petroleum spirits, whereas the secretions of the sebaceous gland have a low solubility in water (Ryder and Stephenson 1968). It is premature at this stage to suggest the chemical structure of the pheromones but the presence of pheromone in the two extracts of the wool and its uptake into petroleum jelly should facilitate its identification.

In Experiment 4 (Table 1) teasing with Dorset rams stimulated the ewes to ovulate ($P < 0.001$) but teasing with testosterone-treated wethers, or their wool, or testosterone-treated ewes, failed to stimulate the ewes. By contrast, Fulkerson *et al.* (1979) who used the same androgen and injection schedule in wethers, reported that 42% of the ewes teased with such treated wethers ovulated, compared with 32% of the ewes teased with rams and 5% of the ewes teased with untreated wethers. Androgens other than testosterone may be involved, or the different responses may be due to breed, as we used Romney wethers while Fulkerson *et al.* (1979) used Merinos. Recent work in New Zealand has shown that Romney rams are less effective than Dorset rams at stimulating anovular ewes to ovulate or commence oestrous activity, with Merino rams being intermediate (Tervit *et al.* 1977; Meyer 1979). These differences are not due to higher plasma testosterone concentrations in the rams since they were higher in Romney than Dorset rams over the teasing period (Tervit and Peterson 1978). Thus factors other than androgens can influence the production of the pheromones, although a threshold level of androgens may be required.

The identification and synthesis of the pheromone(s) could result in a cheap and simple method for inducing a partial synchronisation of first oestrus in a high proportion of ewes. This could be used to facilitate AI or reduce the period of time required to "flush" ewes with expensive supplements.

THE HORMONAL RESPONSES TO TEASING

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Some of the results presented here are from very recent experiments, unpublished when this manuscript was being prepared. Due to a shortage of space, all the experimental details, although available, cannot be included. This paper represents the state of our thinking on the endocrine mechanisms behind the teasing response.

In the normally cycling ewe, the sequence of endocrine events leading to ovulation is 1) decrease in progesterone secretion, 2) increase in basal LH secretion, 3) increase in oestradiol secretion and 4) positive feedback leading to the preovulatory surges of LH and FSH, and ovulation (Baird and Scaramuzzi 1976). Each of these is dependent on the event preceding it. In the seasonally anoestrous or anovular ewe, the sequence cannot begin because there is no corpus luteum, or cannot proceed because rising oestradiol levels exert a strong negative feedback on LH secretion, inhibiting the basal rise prior to the surge (Legan *et al.* 1977). However, introducing rams can induce ovulation in these ewes, so the mechanism of anoestrus can be by-passed or reversed.

The ram-stimulated ovulation is the result of an apparently normal preovulatory surge of LH, which can be very rapidly induced, e.g. half of the ewes in the study by Oldham *et al.* (1978) had their surge within 20h of them being introduced to rams. This rapid response led to the postulate that some, if not all, preovulatory surges are induced by a neural reflex (Oldham *et al.* 1978; Knight *et al.* 1978; Poindron *et al.* 1980). In this reflex, the ram stimulus, acting at hypothalamic level, would bypass the positive feedback action of oestradiol and directly elicit an LH surge. To investigate this possibility the most obvious avenue was to measure oestradiol levels prior to the LH surge. However, this is difficult due to the low levels of oestrogens in the non-pregnant ewe, and the consequent necessity for chromatographic steps in the assay (Scaramuzzi and Land 1976). Indeed, this probably explains the lack of any pattern in peripheral levels of oestradiol reported by Knight *et al.* (1978). In the absence of a sufficiently sensitive assay for peripheral oestradiol, we chose to investigate events closer to the introduction of rams. If there were a pre-ovulatory rise in oestradiol it would most likely be due to increased basal LH secretion as in the normal oestrous cycle (Baird and Scaramuzzi 1976). There was some evidence of an increase in mean levels of LH after the introduction of rams, though it apparently was not associated with ovulation (Chesworth and Tait 1974). Furthermore, LH secretion is pulsatile (Scaramuzzi and Martensz 1975) so there should be an increase in pulse frequency some time after the introduction of rams. We observed such an increase and found that ovulation does not result without it (Martin *et al.* 1980; Poindron *et al.* 1980). The ovary is able to secrete oestradiol after each LH pulse during anoestrus (Scaramuzzi and Baird 1976) so presumably there was a sustained increase in oestradiol levels leading to the initiation of the preovulatory surge of LH.

Further evidence of the necessity for positive feedback has come from studies on the effects of chronic and acute progesterone treatment: luteal phase levels of progesterone (from implants) begun four days prior to the introduction of rams and continued for a further four days (until laparoscopy) prevent

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ovulation; similar treatment begun six hours after the introduction of rams and after the rise in LH pulse frequency, and presumably oestradiol, also prevents ovulation; an injection of progesterone (10 mg in oil) concurrent with the introduction of rams, attenuates but does not prevent the increase in LH pulses, and delays, but does not prevent, the preovulatory surge (Fig. 1). These results indicate that progesterone can prevent ovulation by selectively blocking the LH surge, as in ovariectomized ewes (Scaramuzzi *et al.* 1971). Preovulatory surges resulting from 'reflex' neural action are therefore unlikely, so alternative explanations for the rapidity of the response are needed. Some of the increases in pulsatile LH secretion may themselves be sufficient to induce ovulation (Martin *et al.* 1980). Furthermore, the presence of rams advances the onset and extends the duration of the LH surge induced in ovariectomized ewes with an injection of oestradiol. The presence of rams has also been shown to advance the LH surge in normally cycling entire ewes (Lindsay *et al.* 1975b), so may increase the sensitivity of the ewe's hypothalamus to positive feedback.

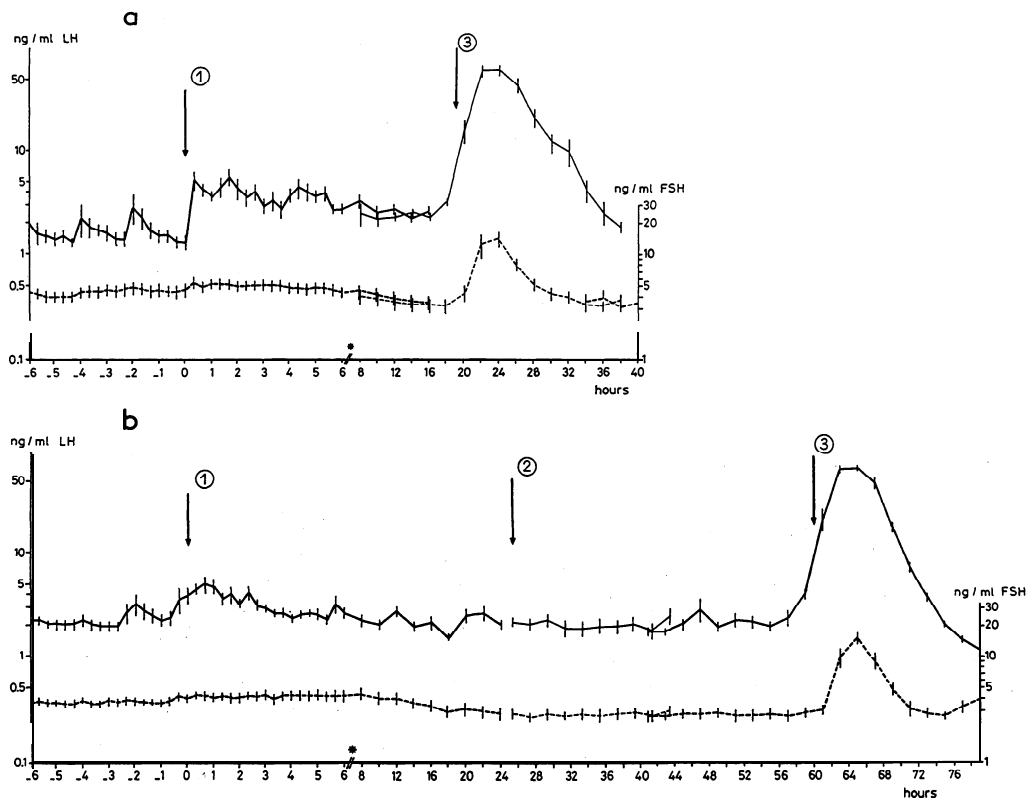


Fig. 1 Mean ($\pm$ SE) levels of LH (—) and FSH (---) in serum of PréAlpes ewes after a) teasing (n=8), or b) teasing plus 10 mg progesterone (n=10). The data are adjusted to 1) introduction of rams, 2) the mean time at which progesterone concentrations returned to < 0.2 ng/ml, or 3) the start of the preovulatory surge of LH. Samples were taken at 20 min intervals until + 6h, and two-hourly thereafter, and assayed according to Poindron *et al.* (1980).

Initially we interpreted the increase in the frequency of LH pulses entirely in terms of 'escape' from negative feedback, since heightened sensitivity to oestradiol has been proposed as the mechanism causing anovulation (Scaramuzzi and Baird 1976; Legan *et al.* 1977). Oestradiol certainly can reduce the frequency of LH pulses (Diekman and Malvern 1973) and a large dose (100 µg oestradiol-17β i.m.) just prior to the introduction of rams will depress and delay the ovulatory response (Martin 1980), while 10 µg oestradiol benzoate i.m. will prevent the rise in frequency of LH pulses. Both of these doses are capable of eliciting positive feedback in anoestrous ewes (Goding *et al.* 1969) making interpretation of the results for LH surges and ovulation difficult, but the effects on basal LH secretion and delayed ovulation were quite clear.

Changes in the frequency of LH pulses, independent of endocrine feedback systems, may be a component of the ram effect. In 3/10 ovariectomized ewes without steroid pretreatment, the frequency rose from 0.6 to 0.9 pulses/h, with a concomitant rise in basal levels, after the introduction of rams. Ovariectomized ewe lambs display a rise in frequency of LH pulses at about the expected time of puberty (D.L. Foster 1980, pers. comm.) and ovariectomized adult ewes demonstrate seasonal changes in pulse frequency without steroid pretreatment (P.J. Wright 1980, pers. comm.). The pulse frequency in ovariectomized animals has been considered maximal but, in the absence of any convincing evidence of the role of adrenal steroids in negative feedback, the data support the thesis that the brain has primary control of pituitary output - not the ovary.

Upon the introduction of rams there are no functionally related changes in the basal levels of FSH in either seasonally or lactationally anovular ewes (Poindron *et al.* 1980) or in ovariectomized ewes. Indeed, in ovariectomized ewes with or without rams, no significant changes were observed after the administration of oestrogen or progestagen, though both steroids had profound effects on basal LH secretion. Furthermore, ewes actively immunized against androstenedione, in which FSH levels are depressed while the frequency of LH pulses is increased (Martensz and Scaramuzzi 1979), show normal responses to the introduction of rams, in terms of ewes ovulating, though the ovulation rate is elevated. This result, the lack of any functionally-related changes in plasma levels, the non-pulsatile form of secretion, and the long half-life, indicate that the role of FSH is primarily permissive.

Prolactin has been implicated in the control of both seasonal and lactational anoestrus (Thimonier *et al.* 1978; Kann *et al.* 1977). However, ewes in either condition will ovulate after the introduction of rams with similar responses in pulsatile LH secretion and ovulation, despite large differences in prolactin levels. Depressing prolactin secretion with a dopamine agonist also has no effect on the response to rams. The frequency of LH pulses was lower in ewes suckling twin lambs than in ewes with single lambs (before the introduction of rams) but this also had no apparent effect on their response to the rams (Poindron *et al.* 1980). Although anovulation and elevated levels of prolactin are both caused by season and lactation, this does not mean that prolactin causes anovulation.

In-conclusion, it seems likely that the primary endocrine response induced in anovular ewes by the introduction of rams is the rapid rise in the frequency of LH pulses. This begins the normal sequence of events which lead to ovulation. The roles of prolactin and FSH are not clear, but changes in the levels of these gonadotrophins are probably not important in determining whether the ewe will ovulate.

INCREASED OVULATION RATE AT THE RAM-INDUCED OVULATION
AND ITS COMMERCIAL APPLICATION

Y. COGNIE, F. GAYERIE, C.M. OLDHAM and P. POINDRON

There is evidence that the ovulation rate (number of ovulations per ewe ovulating) of successfully teased ewes was increased relative to that of their second ovulation or relative to spontaneously ovulating flock mates. The effect was observed in Merino, Préalpes and Ile-de-France breeds and may have been prevented in ewes primed with progestagen before the introduction of rams (Oldham and Cognie, unpublished). Progestagen priming ensures oestrus at the ram-induced ovulation, and ensures that the CL persist for a normal period (Hunter *et al.* 1971; Oldham *et al.* 1980). All of these factors will affect commercial application of teasing. The effects of teasing and progesterone on ovulation rate were tested in experiments on three breeds of ewe, and the possibility of commercial application of teasing to an intensive breeding system was tested in a fourth experiment. Progesterone was administered over 12 days by injections in Experiments 1 and 3 (10 mg/day) or by intravaginal sponges impregnated with fluorogestone acetate (FGA) in Experiments 2 and 4.

RESULTS

Experiment 1. In Western Australia, successfully teased Merino ewes had more twin ovulations than spontaneously ovulating flockmates with the same average live weight ($P < 0.01$, Fig. 2). Among the teased ewes, those primed with progesterone had less twin ovulations than unprimed controls (4/18 vs 10/29) but the difference was not significant. The percentage of twins was high only at the teased ovulation, and fell to control levels at the next ovulation (Oldham 1980).

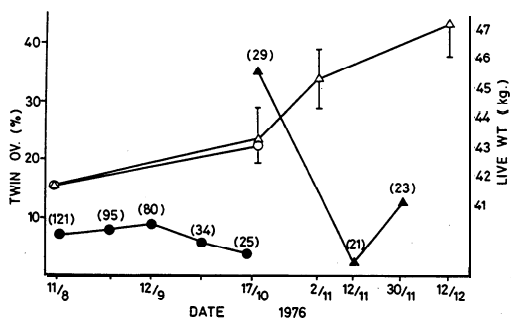


Fig. 2. Live weight (mean $\pm$ SE) and the incidence of twin ovulations (%) among Merino ewes (Exp. 1) ovulating spontaneously or at the ram-induced and subsequent ovulations in successfully teased ewes. Figures in parentheses are the number of ewes ovulating.

Ewes ovulating spontaneously -

●-● % twins

○-○ live weight

Successfully teased ewes -

▲-▲ % twins

△-△ live weight

Experiment 2. In France, the ovulation rate of successfully teased Préalpes ewes was 1.58 compared with 1.31 in unteased controls ($P < 0.05$). Again, the ovulation rate at the second ovulation after teasing fell to control levels. Progesterone priming did not influence ovulation rate.

Experiment 3. In Ile-de-France ewes, teasing increased the mean ovulation rate from 1.54 to 2.15. The progestagen used in this experiment (fluorogestone acetate, FGA) did not affect the proportion of ewes ovulating but had a marked effect on ovulation rate. In FGA-primed ewes the ovulation rate at teasing was only 1.09.

Experiment 4. Results are shown in Table 2. Teasing to induce ovulation, after FGA priming, substituted identically with a dose of PMSG (530 IU) when the results are measured by ovulation rate, fertility, prolificacy or the number of lambs marked. In this experiment, with Berrichon ewes in France, FGA apparently did not depress ovulation rate, as it did in Experiment 3 where Ile-de-France ewes were used. No reason for this discrepancy can be advanced.

CONCLUSION

There is a commercially significant increase in ovulation rate when ewes are teased, which is probably not affected by the progesterone priming that is necessary if the ewes are to mate and conceive. This high ovulation rate can be used to advantage in intensive breeding programmes, and may also be useful in less intensive systems such as those used in Australia.

TABLE 2 Reproductive performance of Berrichon ewes injected with 530 IU PMSG and artificially inseminated (-RAM), or teased and hand-mated (+RAM) after withdrawal of FGA sponges (June 1980)

TR	n	OR					FERTILITY			PROLIFICACY ¹				LAMBS TAILED --	
		Distribution ²					Ewes preg. day 18	Ewes lambing	Distribution ³						
		%	1	2	3	4			%	%	%	1	2	3	4
(-RAM)	15	200	3	9	3		33	81.9	78.7	177	13	9	1	3	158
(+RAM)	15	210	1	12	1	1	30	80.0	76.6	161	10	12	1		157

1. lambs born per ewe lambing.

2. number of ewes with 1, 2, 3 or 4 CL.

3. number of ewes with 1, 2, 3 or 4 lambs.

USE OF TEASING IN AN A.I. PROGRAMME

D.G. CORKE*

Introducing rams to a flock of Merino ewes in November in Western Australia results in two peaks of oestrous activity about 19 and 25 days later. This is caused by some ewes having a six-day cycle immediately after teasing, then a normal 17-day cycle, while others have the normal cycle only (Oldham and Martin 1979).

For synchronizing oestrus in an A.I. programme, the two peaks partially nullify the benefits of teasing. To make the best use of teasing, and to even out the daily work load, rams were introduced to half of the flock on one day and the other half three days later. This should provide two sets of peaks of oestrus which are out of phase and overlapping.

In 1976, joining of teasers was not staggered, and the number of ewes in oestrus varied from 56 to 553 per day (Fig. 3). In 1977, after staggered teasing, the range was 100-327 ewes per day and oestrus was more evenly distributed about the mean of 221 ewes per day. In 1978 and 1979, teasing was also staggered, and similarly produced even numbers of ewes in oestrus each day. Although the

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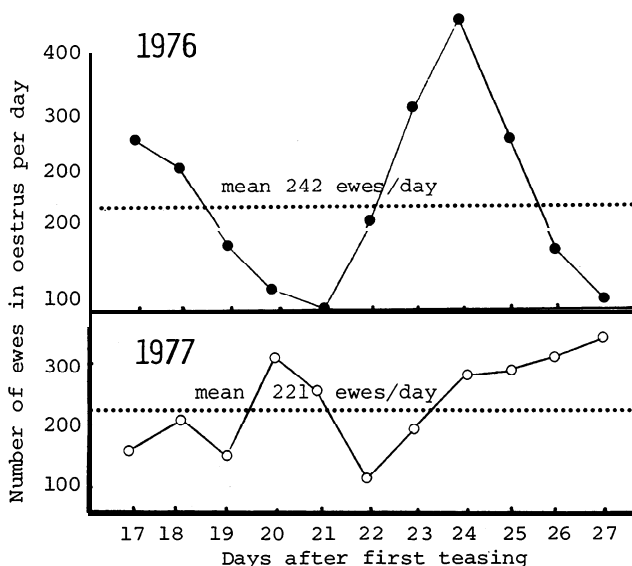


Fig. 3. Effects of staggered teasing on the daily distribution of ewes in oestrus.

comparison between 1976 and 1977 is confounded by year differences, the effects of staggered teasing are repeatable.

Eighty-three to 85% of ewes were artificially inseminated by the eleventh day in 1976, 1977 and 1978. The A.I. programme was therefore not extended beyond 11 days. In 1979, teasing was less effective at inducing oestrus and only 56% of ewes were marked in 13 days. No reason for this can be advanced.

Teasing is useful for synchronizing oestrus and, if staggered, provides a relatively constant work load in an A.I. programme.

DO EWES CONTINUE TO CYCLE AFTER TEASING?

C.M. OLDDHAM and Y. COGNIE

More than half of the Merino ewes in Western Australia are joined between September and January during the last half of their non-breeding season, and of those which mate, about 24% apparently conceive then fail to lamb (Knight *et al.* 1975; Oldham 1980). Ewes which are successfully teased (i.e. ovulate in response to the introduction of rams), cycle once or twice then re-enter anoestrus before conceiving, would fit this category of reproductive wastage. It was proposed to test whether ewes do rapidly return to anoestrus and therefore contribute to reproductive inefficiency.

EXPERIMENTAL

Experiment 1. Merino ewes (Perth, W.A.) were used and, for this experiment only, the control ewes were continuously associated with rams. Ewes which return to anoestrus were those which ovulated in response to the introduction of rams, then become anovular again before the onset of the normal breeding season.

Experiments 2 and 3 were both conducted at Nouzilly (France) with Préalpes and Île-de-France ewes respectively. Ovarian activity was monitored by regular

endoscopy (Oldham et al. 1976a) or by analysis of progesterone levels in plasma samples taken twice weekly (Thimonier 1978). Ewes were considered to be seasonally anovular if they had low (< 1 ng/ml) levels of progesterone or no corpus luteum for at least 17 days. The control groups were kept in isolation from rams, while the treated groups were teased. The number of ewes ovulating was monitored at each cycle. After the initial teasing, in Experiment 3, the rams were removed for two weeks, then re-introduced to tease the ewes a second time.

Experiment 4. A commercial flock of 1,000 mature Merino ewes was isolated from rams in August (Western Australia). Beginning on October 25, random samples of 50 ewes were drawn weekly for 14 weeks from the flock and placed with harnessed vasectomized rams. Crayon marks were recorded and crayon colours were changed weekly. The vasectomized rams were exchanged for harnessed entire rams on January 24 and the last record of oestrus was taken on February 7. To analyse the data, the following assumptions were made: (a) ewes first marked between days 0-14 were cycling spontaneously at teasing, (b) ewes not marked between days 0-28 were not cycling spontaneously and were not stimulated by teasing, (c) ewes first marked between days 14-28 were successfully teased, (d) ewes marked in successive 7-day periods were in oestrus on the day the crayon colour was changed, (e) ewes cycling continuously were those marked during 7-day periods separated by at least one, but not more than two periods, (f) ewes experiencing discontinuous cycles were those marked during 7-day periods separated by more than two periods (cycle length > 28 days).

RESULTS AND DISCUSSION

Experiments 1, 2 and 3 (Fig. 4). In all three experiments, a significant proportion of successfully teased ewes rapidly became anovular again, particularly the Ile-de-France ewes teased in the middle of their non-breeding season (Experiment 3). By contrast, some ewes teased towards the end of their non-breeding season continued to cycle regularly through to the start of their spontaneous breeding season, while 50-60% of their flockmates experienced a short period of anovulation.

In Experiment 1 (Fig. 4a), 20, 30 and 35% of the ewes were marked by the rams once, twice or three times during the first 60 days after teasing, and 15% were not marked at all. If the ewes had been joined with entire rams instead of vasectomised rams, and if the conception rate were, say, 60%, at least 18% of those ewes mated in the first eight weeks of joining would have failed to return to service, and failed to lamb. These ewes returned to anoestrus and did not begin their normal breeding season until after the rams were removed.

Experiment 4 (Fig. 5). The percentage of ewes marked within the first 28 days was constant for each sample (c. 85%), independent of the date of joining, and was composed of a variable proportion of ewes cycling spontaneously (2-25%) and a highly repeatable response to teasing. Most of the ewes in the last sample joined on January 24 were still in anoestrus, but the trend indicates that the spontaneous breeding season was about to begin.

Of successfully teased ewes, only about 35% again became anoestrous, compared with 65% in Experiment 1 which was conducted at the University of W.A. This difference may be a reflection of their past nutritional environment (Smith 1965; Oldham 1980). However, while the proportion of successfully teased ewes re-experiencing anoestrus was relatively uniform between samples, the speed with which ewes returned to anoestrus was related to their date of joining (Fig. 6). The incidence of anoestrus within the first 51 days increases as the date of teasing approaches the start of the new breeding season ($P < 0.01$). This re-

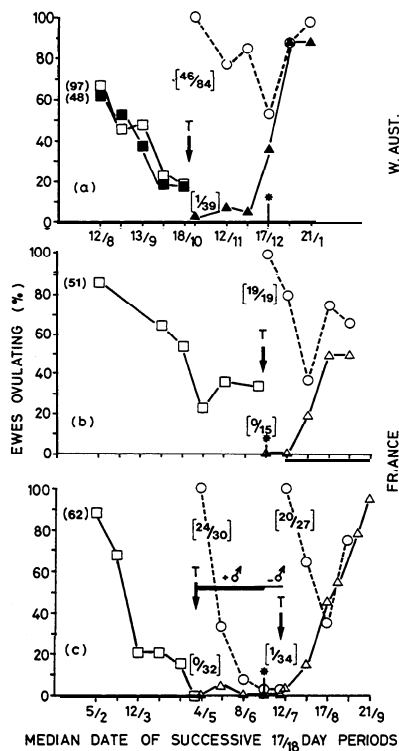


Fig. 4. Proportion of ewes with active CL during successive 17-18 day periods for a) Merinos in Experiment 1, b) Préalpes in Experiment 2, and c) Iles-de-France in Experiment 3.

T teasing (introduction of rams)

() number of ewes in the experiment

[] proportion ovulating

□ ewes isolated from rams

■ ewes continually associated with rams

○ successfully teased ewes

+♂ teasing and presence of rams

-○ re-isolation from rams

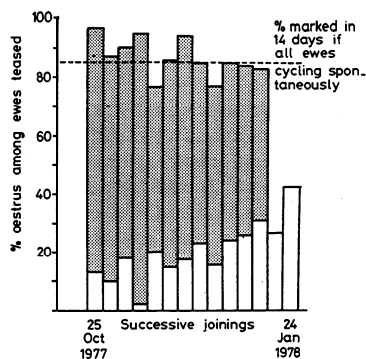


Fig. 5. Incidence of oestrus in samples of ewes drawn from a flock at weekly intervals and teased between late October and late January (Western Australia, Experiment 4).

□ ewes cycling spontaneously at teasing

▨ successfully teased ewes

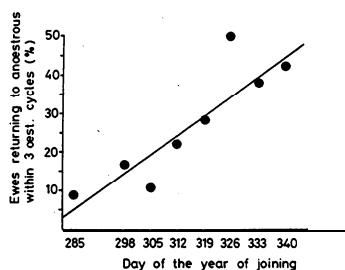


Fig. 6. The relationship between the date of joining and the percentage of successfully teased ewes returning to anoestrus within 3 oestrous cycles (51 days) of joining (Experiment 4)

$$y = -203.43 + 0.73x$$

$$R^2 = 0.79$$

entry of successfully teased ewes to anoestrus is apparently centred on or near the summer solstice. This result contrasts markedly with the ovarian activity of Ile-de-France ewes following teasing in the middle versus the end of their non-breeding season (Fig. 4c). No explanation for this difference can be offered at the moment. Experiment 3 could not be continued through until the start of the flock's spontaneous breeding season to give a complete picture, but it is clear that when Merino ewes are joined between late October and mid-December, successfully teased ewes which fail to conceive run the risk of returning to anoestrus rather than to the ram.

SUMMARY AND CONCLUSIONS

C.M. OLDHAM

Over half of the Merino ewes in Western Australia are joined out of season as are many flocks in eastern Australia and an increasing number of ewes in Europe. Thus, an increased understanding of the physiology of the endogenous mechanism which allows ewes to breed out of season is of fundamental importance.

In addition, the rapid repeatable ovulatory response of seasonally anovular ewes to teasing, coupled with the variable quality of the ram-induced CL and the return of many successfully teased ewes to seasonal anovulation makes it an ideal model for studies into the control of (i) ovulation, (ii) seasonal breeding and (iii) CL quality and function. Despite a large volume of work the mechanisms controlling ovulation rate are still unknown. At teasing there is a transient increase in ovulation rate which is ideal for intensive study.

Scaramuzzi and Baird (1976) and Legan *et al.* (1977) have proposed that a change in the sensitivity of tonic LH secretion to oestradiol controls seasonal breeding. The ram stimulus, then, must reverse the as yet unknown mechanism controlling the change in sensitivity. Following successful teasing, many ewes continue to cycle while others return to anovulation. Why? The answer may help to elucidate the mechanism which controls the sensitivity of LH to oestradiol.

Similar arguments support the use of this system for exploring CL function. Short-life-span CL are observed at puberty (Foster and Ryan 1979) after lactational anoestrus (Land 1971), at the onset of spontaneous ovulation after a period of anovulation (C.M. Oldham and Y. Cognie, unpublished) and at teasing. Why at all these times do some CL persist and function normally while others regress prematurely? What is the mechanism for luteolysis at the premature regression? Does progesterone priming ensure normal CL function? If so, how?

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