Ram induced stimulation of ovarian and oestrous activity in anoestrous ewes — a review

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ABSTRACT

The stimulation of oestrus in ewes early in the breeding season has been known for a number of years but only recently has there been a full understanding of the changes which occur in the ewe. Recent work indicates that only 17 to 21 days isolation from rams is needed to sensitise ewes to the ram. Within 10 minutes of introducing rams, the luteinising hormone (LH) levels increase in the ewe. This can result in a preovulatory LH surge 27 to 35 hours later and a silent ovulation within 54 to 72 hours in anovular ewes. However, 43 to 59% of ewes have a premature regression of the corpus luteum (CL) and a second silent ovulation occurs 4 to 6 days later. This leads to flocks having peaks of first oestrus around days 18 and 24 after ram introduction. Injection of progesterone at ram introduction prevents premature regression of the CL and first oestrus is synchronised over days 19 to 21 after ram introduction. The percentage of multiple ovulations is higher at the silent ovulation than at the first oestrus or in spontaneously ovulating flock mates. Priming with progestagens for 12 to 16 days can induce oestrus at the first ovulation after ram introduction and still maintain the increased proportion of multiple ovulations.

The ram effect is due to pheromones which are present in the ram's wool and wax. The buck is as effective as the ram at stimulating ewes and fatty acid pheromones isolated from the buck are also effective at stimulating ewes.

Keywords Ewes; pheromones; ram stimulation; silent ovulations; anoestrus

INTRODUCTION

New Zealand farmers have been aware that teaser rams will stimulate a synchronised oestrus in ewes early in the breeding season since the work of Schinckel (1954 a) and Edgar and Bilkey (1963). This work indicated that the ewes had a silent ovulation (i.e. ovulation without behavioural oestrus) soon after ram introduction and it was suggested the progesterone from the CL primed the ewes for their first oestrus one cycle later (Schinckel, 1954 a, b). Most breeds of ewes can be stimulated by the ram (Merino; Schinckel, 1954 a, Romney; Edgar and Bilkey, 1963, Awassi; Eyal, 1968, Corriedale; Lyle and Hunter, 1967, Prealpes, Ile-de-France and Berrichon; Cognie *et al.*, 1980).

Further research in the last 5 years has given a clearer understanding of the mechanisms involved in the ram induced ovulation and first oestrus.

Time of Year and Isolation of Ewes

The proportion of ewes ovulating and/or exhibiting oestrus after ram introduction varies greatly and breed of ewe and time of year are 2 major factors influencing the response. Merino ewes will respond to teasing at almost any time during anoestrus (Lishman, 1969), while Romney ewes only respond in late anoestrus, with 10 February being the optimum time (Edgar and Bilkey, 1963).

A prerequisite for a response is that ewes are isolated from rams for a period before ram introduction. Ewes run continuously with rams adapt to their presence and have a level of anoestrus similar to isolated ewes (Riches and Watson, 1954; Lishman, 1969). The minimum period of isolation is unknown but 17 and 34 days isolation were equally effective when compared with non-isolated Merino ewes with 47%, 44% and 3% ovulating after teasing (Oldham, 1980 a). Twenty-one days isolation has been found to be adequate for Ile-de-France ewes (Oldham, 1980 b).

Silent Ovulations and First Oestrus

Schinckel (1954 b) found that ewes have a silent ovulation within 6 days of ram introduction. More recently, using a laparoscope and continued observations on the same ewes, it has been found that 65% of Romney ewes ovulate within 65 hours of ram introduction (Knight *et al.*, 1978) and 50% of Merino ewes within 41 hr (Oldham *et al.*, 1978/9). Similar tight synchronisation is however not seen at first oestrus. There is usually a spread of 10 days with peaks around days 18 and 24 after ram introduction (Schinckel, 1954 a; Eyal, 1958; Lyle and Hunter, 1967). The explanation is that in a proportion of ewes (Romney 59%, Knight *et al.*, 1981, Merino 43%, Oldham and Martin, 1978/9) the CL formed from the silent ovulation regresses after 4 to 6 days and is followed by a second silent ovulation. A schematic

diagram of the events following ram introduction is presented in Fig. 1.

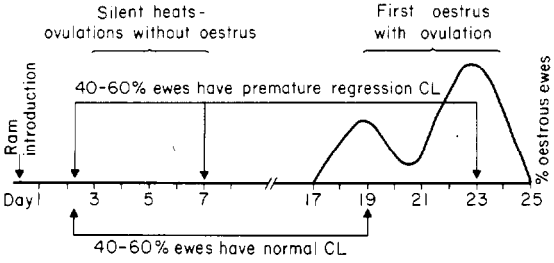

FIG. 1 A schematic diagram of the time after ram introduction of the onset of ovulatory and oestrous activity and the premature regression of the corpus luteum.

Cognie *et al.* (1982) have found that the injection of 20 mg progesterone at ram introduction prevented the premature regression of the CL with 90% of Merino ewes exhibiting oestrus over days 19 to 21 after ram introduction. Similarly, priming ewes with progestagens for 12 to 16 days before ram introduction prevented the premature regression of the CL (Oldham *et al.*, 1980). Oestrus accompanied the first ovulation if ram introduction occurred at sponge removal or the last progestagen injection (Lishman and Hunter, 1966; Hunter *et al.*, 1971, Cognie *et al.*, 1982). If ram introduction is delayed 48 to 52 hours, ovulation occurs but the ewes do not exhibit oestrus. With progesterone priming alone the ewes neither exhibit oestrus nor ovulate (Oldham, 1980 a; Martin *et al.*, 1981).

The proportion of multiple ovulations is higher at the first ovulation than the second (Romney 48% *v* 24%, T. W. Knight, unpublished; Merino 35% *v* 5%, Oldham, 1980 a) and higher than in flock mates that ovulate spontaneously (Merino 35% *v* 4%; Prealpes 58% *v* 31%; Ile-de-France 115% *v* 58%; Cognie *et al.*, 1980). The effect of priming with progestagens for 12 to 16 days on the proportion of multiple ovulations following teasing is variable. In Merino ewes progesterone priming depressed the multiple ovulations from 35% to 19% but it was still higher than the 4% multiple ovulations in the spontaneously ovulating flock mates (Oldham, 1980 a). Similarly, in Ile-de-France ewes teasing increased the mean ovulation rate from 1.54 to 2.15 but priming with fluorogestone acetate depressed the ovulation rate to 1.09 (Cognie *et al.*, 1980). By contrast, in Prealpes and Berrichon ewes progestagen priming did not suppress the increased proportion of multiple ovulations. The rams were equivalent to the use of 530 IU PMSG and similar fertility (80%) and proportion of multiple births were obtained (Cognie *et al.*, 1980).

Hormonal Changes

The luteinising hormone (LH) concentration in anoestrous Merino ewes increased from basal levels of < 1 ng/ml to 2 to 22 ng/ml within 10 minutes of introducing rams (Martin *et al.*, 1980). Not all ewes responded and some of those that did respond did not ovulate. These workers suggest that the increase in LH stimulates oestradiol release which in turn stimulates the preovulatory surge of LH seen 27 ± 4 hours after ram introduction in Merino ewes (Oldham *et al.*, 1978/9); 35 ± 5 hours in Romney (Knight *et al.*, 1978) and 24 to 31 hours in Prealpes (Poindron *et al.*, 1980). However, there is some evidence that the pre-ovulatory LH surge may occur without increased oestradiol (Knight *et al.*, 1978). Both Oldham *et al.* (1978/9) and Martin *et al.* (1980) reported increases in LH similar to the pre-ovulatory surge within 6 to 12 hours of ram introduction. This is too soon for an oestradiol stimulated LH surge (Baird and Scaramuzzi, 1976).

The immediate increase in LH after ram introduction has been found in seasonally anovular Prealpes and Ile-de-France ewes and during the breeding season in lactationally anoestrus Prealpe ewes (Poindron *et al.*, 1980). The LH response to rams has also been observed in ovariectomised ewes treated with oestradiol (Martin and Scaramuzzi, 1981), in ewes in the middle of anoestrus (J-P. Signoret, pers. comm.) and during the breeding season (T. W. Knight, unpublished).

Ewes with premature regression of the CL have small peaks of progesterone on days 4 to 5 but these decline on days 6 to 7 (Fig. 2, Knight *et al.*, 1981).

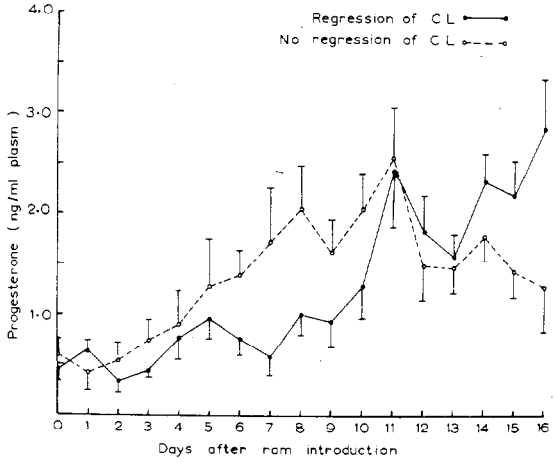

FIG. 2 Progesterone concentrations in ewes that ovulated by day 4 and either had premature regression of the corpus luteum over days 4 to 8 or no premature regression of the corpus luteum. Each point is the mean of 8 ewes (from Knight *et al.*, 1981).

Ram Pheromones

The first evidence that the stimulation of ewes was mediated by ram pheromones was the observation (Watson and Radford, 1960) that anoestrous Merino ewes separated from rams by hessian screens were

stimulated as effectively as ewes in visual and physical contact with rams. Ewes with their sense of smell impaired did not exhibit oestrus after being teased by rams (Morgan *et al.*, 1972).

Knight and Lynch (1980 a) found that urine collected from Dorset rams stimulated only 22% of anovular ewes to ovulate while the wool and wax from the rams stimulated 48%. This was similar to the 43 to 50% stimulated by the Dorset rams and contrasted with the 0 to 7% ovulating in the isolated ewes. Subsequent work (Knight and Lynch, 1980 b; Knight *et al.*, 1983) has confirmed that both the wool and wax from rams contain pheromones which, when applied to anovular ewes 3 times a day for 2 days, will stimulate them to ovulate. The pheromones are present in aqueous and petroleum spirit extracts of the wool and wax and can be absorbed into petroleum jelly that has been smeared over a ram's back for 24 hours (Knight and Lynch, 1980 b).

The steroids 3 α-hydroxy-5α-androst-16-ene and 5α-androst-16-ene-3-one, which are the pheromones produced by boars, failed to stimulate anovular ewes to ovulate. However, goat bucks were as effective as rams at stimulating ewes to ovulate—57 and 75% of ewes ovulated after stimulation with bucks and rams respectively compared with 7% ovulating in the isolated ewes (Knight *et al.*, 1983). In a more recent trial, the fatty acids isolated from buck hair stimulated 50% of anovular ewes to ovulate compared with 40% ovulating after teasing with Dorset rams. These responses were significantly different ($P = 0.016$ and $P = 0.043$ respectively) from the 0% of isolated ewes ovulating (T. W. Knight, E. J. Birch and G. J. Shaw, unpublished). The fatty acids isolated from buck hair consist of a mixture of 4-ethyl-octanoic, -decanoic, -dodecanoic, and -tetradecanoic acids and are produced by the sebaceous glands of the buck. There is no trace of these acids in ram's wool (E. J. Birch and G. J. Shaw, unpublished).

Factors Controlling Pheromone Production

Ewes and wethers treated for short periods with high doses of androgens or oestrogens can be as effective as rams at stimulating ewes to ovulate and exhibit oestrus (Lishman *et al.*, 1969; Fulkerson *et al.*, 1981; Croker *et al.*, 1982). The latter 2 groups of workers treated Merino wethers and ewes 3 times at weekly intervals with either 105 mg testosterone propionate (Tesgro—MSD), 100 mg testosterone cyclopentyl propionate (Banrot—Wellcome Laboratories) or 1 mg oestradiol cypionate.

By contrast, Knight and Lynch (1980 b) found that Romney ewes and wethers treated with 105 mg testosterone propionate (Tesgro—MSD) 3 times at weekly intervals failed to stimulate anovular ewes. When these animals were treated for a further 2 weeks the wethers and ewes stimulated 15% and 17% of ewes to exhibit oestrus compared with 6% of the untreated ewes and 20% of the ewes teased with Dorset rams (T. W. Knight, unpublished). This poor response with the testosterone treated Romney ewes and wethers may reflect the general poor ability of the Romney to stimulate ewes. Tervit *et al.* (1977) first observed that Dorset rams were more effective than Merino rams which in turn were more effective than Romney rams at stimulating ewes. The superiority of Dorset rams over Romneys was confirmed by Knight *et al.* (1980). Since testosterone concentrations over the teasing period are higher in the Romney than Dorset rams (Tervit and Peterson, 1978) other factors besides androgens must be involved. As well as these between breed differences, there is evidence of differences between strains of Dorset rams and between rams in their ability to stimulate ewes. In addition there are suggestions that the ability of rams to stimulate ewes may vary with seasons. This may be related to seasonal variation in testosterone production since teasing anovular Romney ewes on 11 August with testosterone-treated Dorset rams or the wool from these rams, stimulated 60% and 40% of ewes to ovulate compared with none ovulating in both the isolated ewes and ewes stimulated with untreated Dorset rams ($P < 0.05$) (T. W. Knight, unpublished).

If it is assumed that pheromones are the major component of the 'ram effect' the results suggest that pheromones can be produced by both sexes and production is rapidly stimulated by androgens and oestrogens. While testosterone may be the major factor controlling production, the breed and strain differences indicate that other factors are involved.

Duration of Teasing and Rate of Return to Anoestrus

Although LH increases within 10 minutes of ram introduction, rams must remain with the ewes for longer than this to get an ovulatory response (G. B. Martin, pers. comm.). Knight (1980) found that while 24 hours teasing increased the percentage of ewes exhibiting oestrus 17 to 24 days later, 48 hours teasing was required to give a response equivalent to teasing for 17 days.

Merino, Ile-de-France and Prealpe ewes can be stimulated to ovulate several months before the normal breeding season and they rapidly return to anoestrus after stimulation with the rams. Of Merino ewes stimulated to ovulate on 18 October, 22% had become anovular 24 days post teasing and 42% by 50 days. The rate of return to anoestrus in Merino ewes increased as the summer solstice was approached (Oldham, 1980 a; b). This problem does not appear in the Romney ewes because they can only be stimulated by rams 2 to 4 weeks before the onset of the breeding season (Edgar and Bilkey, 1963).

CONCLUSIONS

The recent research into the 'ram effect' has explained

some of the anomalies of earlier work and has provided further practical applications of the 'ram effect'. The injection of progesterone at ram introduction can give a high degree of synchronisation 19 to 21 days later and eliminates the biphasic onset of first oestrus seen in untreated ewes due to premature regression of the CL. The increased ovulation rate at the silent ovulation can be capitalised on by priming with progesterone to stimulate oestrous behaviour. However further work is required to define the optimum time of ram introduction relative to the end of the progesterone priming.

Further work at Whatawhata Research Station is proceeding in conjunction with DSIR and Ruakura, to isolate and identify the ram and buck pheromones. The use of pheromones could overcome the unreliability of the 'ram effect' due to the between breed and between ram differences in ability to stimulate ewes and possible seasonal variations in pheromone production. It may also enable a much earlier stimulation of oestrus in breeds such as the Romney which have a well defined breeding season.

The basic hormonal studies of the 'ram effect' have provided new insights into the factors controlling ovulation, CL function and oestrus.

REFERENCES

Baird D. T.; Scaramuzzi R. J. 1976. Changes in the secretion of ovarian steroids and pituitary luteinising hormone in the periovulatory period in the ewe: effect of progesterone. *Journal of endocrinology* **70**: 237-245.

Cognie Y.; Gayerie F.; Oldham C. M.; Poindron P. 1980. Increased ovulation rate at the ram-induced ovulation and its commercial application. *Proceedings of the Australian Society of Animal Production* **13**: 80-81.

Cognie Y.; Gray S. J.; Lindsay D. R.; Oldham C. M.; Pearce D. T.; Signoret J. P. 1982. A new approach to controlled breeding in sheep using the 'ram effect'. *Proceedings of the Australian Society of Animal Production* **14**: 519-522.

Croker K. P.; Butler L. G.; John M. A.; McColm S. C. 1982. Induction of ovulation and cyclic activity in anoestrous ewes with testosterone treated wethers and ewes. *Theriogenology* **17**: 349-354.

Edgar D. G.; Bilkey D. A. 1963. The influence of rams on the onset of the breeding season in ewes. *Proceedings of the New Zealand Society of Animal Production* **23**: 79-87.

Eyal E. 1958. The introduction of rams as a factor influencing sexual activity of ewes. *Israel journal of agricultural research* **8**: 359-366.

Fulkerson W. J.; Adams N. R.; Gherardi P. B. 1981. Ability of castrate male sheep treated with oestrogen or testosterone to induce and detect oestrus in ewes. *Applied animal ethology* **7**: 57-66.

Hunter G. L.; Belonji P. C.; Van Niekerk C. H. 1971. Synchronised mating and lambing in spring-bred Merino sheep. The use of progestagen-impregnated intravaginal sponges and teaser rams. *Agroanimalia* **3**: 133-140.

Knight T. W. 1980. Onset of mating activity in Romney ewes after short periods of teasing. *New Zealand journal of agricultural research* **23**: 277-280.

Knight T. W.; Dalton D. C.; Hight G. K. 1980. Changes in the median lambing dates and lambing pattern with variation in time of joining and breed of teasers. *New Zealand journal of agricultural research* **23**: 281-285.

Knight T. W.; Lynch P. R. 1980 a. Source of ram pheromones that stimulate ovulation in the ewe. *Animal reproduction science* **3**: 133-136.

Knight T. W.; Lynch P. R. 1980 b. The pheromones from rams that stimulate ovulation in the ewe. *Proceedings of the Australian Society of Animal Production* **13**: 74-76.

Knight T. W.; Peterson A. J.; Payne E. 1978. The ovarian and hormonal response of the ewe to stimulation by the ram early in the breeding season. *Theriogenology* **10**: 343-353.

Knight T. W.; Tervit H. R.; Fairclough R. J. 1981. Corpus luteum function in ewes stimulated by rams. *Theriogenology* **15**: 183-190.

Knight T. W.; Tervit H. R.; Lynch P. R. 1983. The effect of boar pheromones, ram's wool and presence of bucks on anovular ewes early in the breeding season. *Animal reproduction science* (in press).

Lishman A. W. 1969. The seasonal pattern of oestrus amongst ewes as affected by isolation from and joining with rams. *Agroanimalia* **1**: 95-102.

Lishman A. W.; Hunter G. L. 1966. Effect of progesterone treatment prior to joining breeding rams, on the incidence of oestrus and rate of lambing in a Merino flock. *Proceedings of the South African Society of Animal Production* **5**: 137-139.

Lishman A. W.; de Lange G. M.; Filjoeu J. T. 1969. Ability of masculinised ewes to stimulate onset of the breeding season in maiden Merino ewes. *Proceedings of the South African Society of Animal Production* **8**: 140-141.

Lyle A. D.; Hunter G. L. 1967. Teasing groups of ewes at staggered intervals as a means of levelling the ram mating load and flock lambing rate. *South African journal of agricultural science* **10**: 597-608.

Martin G. B.; Oldham C. M.; Lindsay D. R. 1980. Increased plasma LH ewes in seasonally anovular Merino ewes following the introduction of rams. *Animal reproduction science* **3**: 125-132.

Martin G. B.; Scaramuzzi R. J. 1981. Pulsatile secretion of LH in ovariectomised ewes: effects of oestradiol and the introduction of rams. *Proceedings of the Australian Society of Reproductive Biology* **13**: 10.

Martin G. B.; Scaramuzzi R. J.; Lindsay D. R. 1981. Induction of ovulation in seasonally anovular ewes by the introduction of rams: effects of progesterone and active immunisation against androstenedione. *Australian journal of biological science* **34**: 569-575.

Morgan P. D.; Arnold G. W.; Lindsay D. R. 1972. A note on the mating behaviour of ewes with various senses impaired. *Journal of reproduction and fertility* **30**: 151-152.

Oldham C. M. 1980 a. A study of sexual and ovarian activity in Merino sheep. PhD Thesis, University of Western Australia, Perth.

Oldham C. M. 1980 b. Stimulation of ovulation in seasonally or lactationally anovular ewes by rams. *Proceedings of the Australian Society of Animal Production* **13**: 73-85.

Oldham C. M.; Cognie Y.; Poindron P.; Gayerie F. 1980. *Proceedings 9th International Congress on Animal Reproduction and Artificial Insemination*: 42.

Oldham C. M.; Martin G. B. 1978/9. Stimulation of seasonally anovular Merino ewes by rams. II. Premature regression of ram-induced corpora lutea. *Animal reproduction science* **1**: 291-295.

Oldham C. M.; Martin G. B.; Knight T. W. 1978/9. Stimulation of seasonally anovular Merino ewes by rams. I. Time from introduction of the rams to the pre-ovulatory LH surge and ovulation. *Animal reproduction science* **1**: 283-290.

Poindron P.; Cognie Y.; Gayerie F.; Orgeur P.; Oldham C. M.; Ravault J. 1980. Changes in gonadotrophins and prolactin levels in isolated (seasonally or lactationally) anovular ewes associated with ovulation caused by the introduction of rams. *Physiology and behaviour* **25**: 227-236.

Riches J. H.; Watson R. H. 1954. The influence of the introduction of rams on the incidence of oestrus in Merino ewes. *Australian journal of agricultural research* **5**: 141-147.

Schinckel P. G. 1954 a. The effect of the ram on the incidence and occurrence of oestrus in ewes. *Australian veterinary journal* **30**: 189-195.

Schinckel P. G. 1954 b. The effect of the presence of the ram on the ovarian activity of the ewe. *Australian journal of agricultural research* **5**: 465-469.

Tervit H. R.; Havik P. G.; Smith J. F. 1977. Effect of breed of ram on the onset of the breeding season in Romney ewes. *Proceedings of the New Zealand Society of Animal Production* **37**: 142-148.

Tervit H. R.; Petersen A. J. 1978. Testosterone levels in Dorset and Romney rams and the effectiveness of these breeds in stimulating early onset of oestrus in Romney ewes. *Theriogenology* **9**: 271-277.

Watson R. H.; Radford H. M. 1960. The influence of rams on onset of oestrus in Merino ewes in the spring. *Australian journal of agricultural research* **11**: 65-71.