

Controlled Sheep Breeding: Update 1980-1985*

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Abstract

This contribution to the Symposium concerns four topics which have been addressed in our laboratory over the past five years.

First, the responses to a controlled light environment of Merino ewes and rams have been compared with those of two British breeds. The endocrinological patterns were similar in all breeds but cyclic ovarian activity and ram libido were different. While showing a degree of entrainment to photoperiod, the breeding patterns were much less rigidly controlled in the Merinos than in the others.

Second, the effectiveness of establishment of a cervical reservoir of spermatozoa, in ewes in which oestrus and ovulation have been controlled, has been re-examined. This is highly dependent on the time of insemination relative to that of the release of LH. Maximum numbers are found when ewes are inseminated shortly after the LH peak, i.e. some 6-10 h after the onset of oestrus.

Third, the quantitative and temporal endocrinological and behavioural events following standard, progestagen - PMSG treatment have been quantified. Contrary to earlier expressed beliefs, these events are remarkably predictable provided an intensive system of mating or detection of oestrus is used. The onset of oestrus in treated anoestrous crossbred ewes has a normal distribution, with a range of 24 h, centred around a mean of 33 h after withdrawal of a 30 mg Cronolone intravaginal sponge and injection of 500 i.u. PMSG. This period of time is dose-dependent. The LH peak occurs 4.5 ± 0.7 h later and the times of onset of oestrus and of LH release are highly correlated ($r = 0.93$). Ovulation is some 24 h later again.

Fourth, differences in the response of ewes to different batches of PMSG have been defined. While the three commercial preparations studied regularly induced ovulation in anoestrous ewes at doses of 250 i.u. and above, the quantitative responses varied greatly. One preparation would not induce multiple ovulation, even at high doses. There are differences in steroidogenesis and in pregnancy rates, associated with dose of PMSG and the consequent ovulation rate: the ideal would be for every ewe to shed two or three ova. A higher ovulation rate is acceptable, as early embryonic mortality generally reduces the litter size. This is particularly important in deep anoestrus. However, this does not solve the problem of breeding in early lactation.

Introduction

In my Hammond Memorial Lecture of December 1980 (Robinson 1982), I stated: 'This is where we now stand. Controlled year-round breeding of sheep and goats is in commercial use internationally, despite the requirement for excessive sperm numbers. However, to improve the technique and gain further control over breeding activity, more basic information about the factors which control the breeding season and the ovarian and oestrous cycles is needed. In short, sheep and goat breeding technology has been taken to the limit of current scientific knowledge.'

At the time, I posed four questions, each of which involved an important area for investigation. What follows is an account of our progress within these areas during these past five years.

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Areas of Investigation — The Four Questions

Response to Photoperiod of the Merino and British Breeds

Although the sheep is referred to as a 'short-day breeder', the Merinos of Australia and South Africa are joined successfully in the summer when the days are long. Admittedly, they show seasonality: for example, maximum ovulation rates occur in the autumn, coincident with the peak of reproductive activity of more seasonal breeds (Dun *et al.* 1960), but they still can be bred in summer. So the question as to whether or not the Merino differs fundamentally from the more seasonal breeds in its response to photoperiod has been addressed.

Prior to 1980, it had been shown that the pattern of endocrinological changes to photoperiod of the Merino was similar to that of its crosses with British breeds (Evans and Robinson 1980a, 1980b), so the next step was to compare the Merino with pure-bred British breeds known to exhibit a high degree of seasonality. To this end, an experiment was set up in which four rams and four ewes from each of the three breeds, Romney Marsh, Dorset Horn and Merino, were subjected to a series of 'years' of 8 months or 6 months duration consisting of equal periods of 'long' (16 h light : 8 h dark) and 'short' days (8 h light : 16 h dark). Following a settling-in period of 16 weeks of 12 h light : 12 h dark, the regimes of short or long days were commenced with the two rooms out of phase. This was to eliminate extraneous influences, including temperature (Poulton and Robinson 1987). Measurements included scrotal sac volume, plasma testosterone concentration and libido in the rams, and ovarian activity, as measured by twice weekly plasma progesterone assays, in the ewes. The reaction patterns to the regimes of long and short days were indistinguishable in both rooms, so the data were pooled.

Fig. 1 shows that scrotal sac volume and plasma testosterone concentration of the rams showed a cyclic pattern of growth and regression, influenced by the light regime. Maximum activity was attained 15–19 weeks after the switch from short to long days, regardless of whether the imposed 'year' was of 32 or 24 weeks duration, so that increase in both measurements occurred during long days and decrease during short days. The frequency of the curves was that of the duration of the imposed 'year', with a four-month time lag, and the amplitude was a function of the duration of each phase of long or short days. It was a clear case of entrainment, which I have referred to as a biological servo-system (Robinson 1982). There was no significant difference between breeds. Sexual behaviour also was cyclic but, as can be seen from Fig. 1, peak activity lagged some 6–7 weeks behind peak testicular size and testosterone production. Further, there was a highly significant breed effect: the libido of the Merinos was consistently higher than that of the Romneys. Even when the testes were at their most regressed, the libido of the Merinos was as high as that of the Romneys at their peak, despite no demonstrable difference in plasma testosterone concentration.

Fig. 2 shows the reaction pattern for the ewes. All of the Romney and Dorset ewes showed marked seasonality, related to the imposed light regime, whereas only one of the Merinos did so. Interestingly, the mean peak of sexual activity lagged behind that of maximum scrotal sac volume of the rams kept in the same environment. We had observed this earlier (Evans and Robinson 1980b) and it raises an interesting biological point. Lincoln and Davidson (1977) reported a similar delay between maximal scrotal sac volume and aggressiveness in Soay rams, so it is not an artifact. Also the delay corresponds to the duration of the spermatogenic cycle (Ortavant 1959). One could postulate, therefore, that peak numbers of spermatozoa would be available at the time of maximum libido, which happens also to be the peak of cyclic ovarian activity in the ewe. If this can be substantiated, we have here a beautifully timed biological mechanism.

This is a nice tidy model in so far as the Romneys and the Dorsets are concerned, but not for the Merinos. While all four rams followed the same general cyclic patterns as did those of the other breeds, only one of the four ewes did so.

Endocrinological Requirements for the Establishment of Sperm Reserves in the Ewe

This vexed question was first tackled almost 20 years ago by Allison and Robinson (1970,

1972) and was re-addressed in the late 1970's. The hypothesis was that the plasma progestagen profile following withdrawal of an artificial regime differs from that of normal luteolysis — it is much more precipitate — and that this alters the pattern of subsequent endocrinological

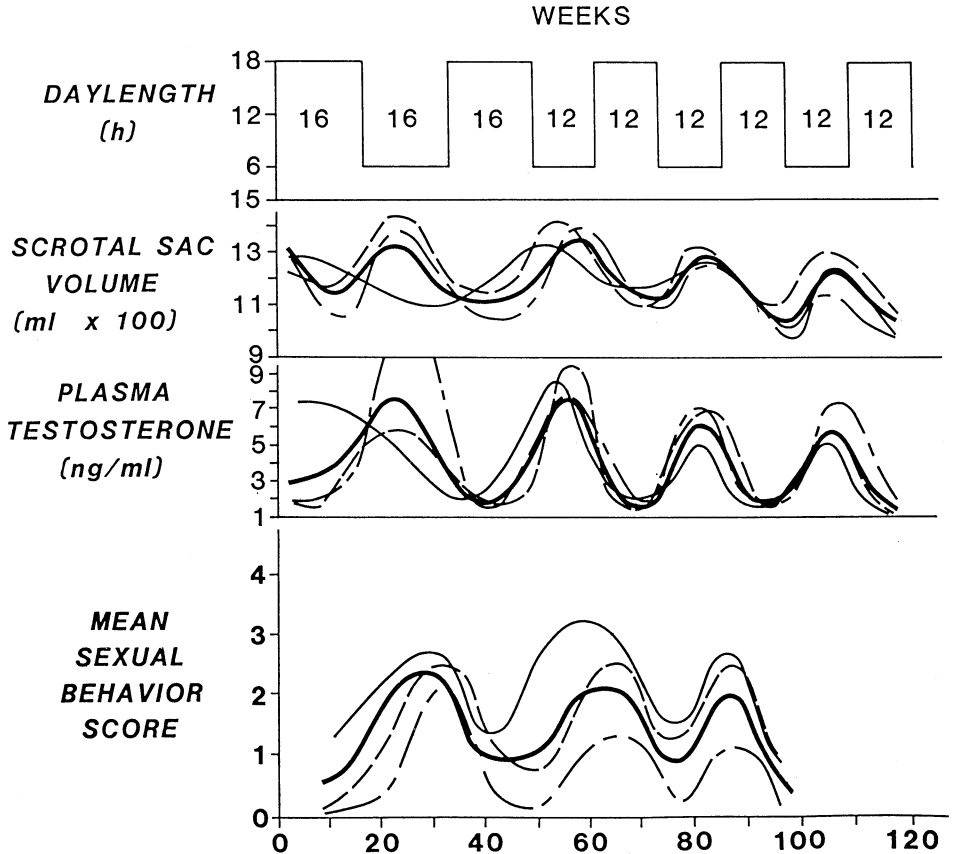


Fig. 1. Mean patterns of response to artificial photoperiod of scrotal sac volume, plasma testosterone and sexual behaviour score of rams of three breeds, Romney Marsh (— — —, $n = 3$), Australian Merino (— — — —, $n = 4$), Dorset Horn (— · — · —, $n = 4$), showing the deviations from the overall mean (———). Only the sexual behaviour score of the three breeds was significantly different ($P \leq 0.001$). (Reproduced from Poulton and Robinson 1987, with permission.)

events. This results in a vaginal, cervical and uterine environment which differs from normal and which is not optimal for the establishment of a large cervical reservoir of spermatozoa.

Interestingly, despite the widespread use, since 1964, of the intravaginal route for the administration of progesterone and some of its analogues (Robinson 1964), no data have been available for plasma concentrations of progesterone or of its most widely used analogues — Cronolone (fluorogestone acetate, FGA: Searle) or Provera (Upjohn), either during or after treatment. We have attempted to redress this situation in several ways.

First of all, we used a radioimmunoassay (RIA) to measure the progesterone profile in cyclic Merino ewes with impregnated intravaginal sponges in place, and then after withdrawal, and attempted to relate the endocrinological events which followed such treatment, as compared with normal luteolysis, with the numbers of spermatozoa recovered from the cervix 2 or 4 h after insemination (Pearce and Robinson 1985). Sponges impregnated with 500 mg progesterone maintained physiological concentrations of plasma progesterone (> 2 ng/ml) for 10 days; by 12 days the concentration had fallen to 1 ng/ml in some ewes. Following withdrawal, the concentration fell rapidly and attained basal levels of < 0.2 ng/ml by 4 h

(Fig. 3). Attempts to alter this profile by the injection of supplementary progesterone at the time of sponge withdrawal were not successful: all we did was induce a brief peak followed by a rapid decline. All other events then occurred sequentially but there were widely differing

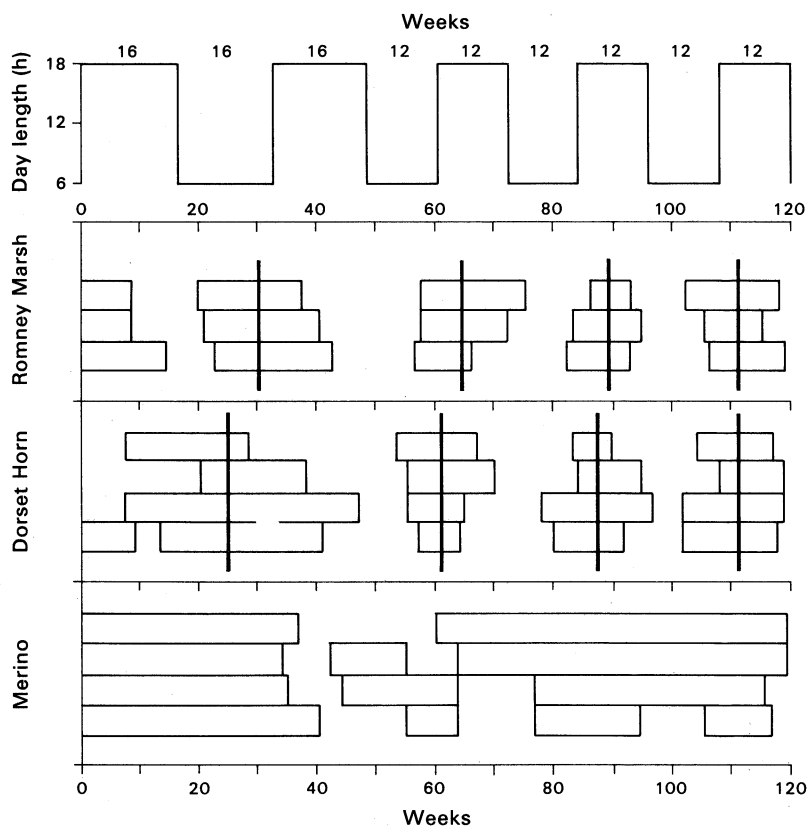


Fig. 2. Mean patterns of response to artificial photoperiod of cyclic ovarian activity, as determined by plasma progesterone concentrations, of ewes of three breeds. The vertical lines indicate the mean centres of activity for the 'seasonal' Romney Marsh ($n = 3$) and Dorset Horn ($n = 4$) ewes. Only one of the Australian Merinos ($n = 4$) was 'seasonal'. (Reproduced from Poulton and Robinson 1987, with permission.)

intervals to oestrus. None of the treatments ameliorated the problem of sperm transport. Numbers of spermatozoa recovered from cyclic ewes were greater than from treated ewes: however, treatment with 400 i.u. PMSG resulted in a significant improvement. Two important observations were made. Although the use of supplementary progesterone resulted in an increase of from 27 to 49 h from sponge withdrawal to the onset of oestrus, the time of the LH surge relative to that of oestrus remained constant, the peak occurring some 5 h after detection of oestrus. The second point was that ewes inseminated 0–8 h after a detected LH surge developed a much larger cervical reservoir than did those inseminated earlier or later (Table 1).

Secondly, we set out to define the plasma profiles provided by Cronolone sponges. The first step was the development of a highly sensitive and specific double antibody RIA for Cronolone. The second was its use to measure the plasma profile of anoestrous ewes treated with intravaginal sponges containing 40 or 80 mg Cronolone (Table 2) during and after treatment. Concentrations of more than 1.0 ng/ml were maintained for 10–12 days, levels much higher than might have been expected given that this progestagen has a physiological activity some 25 times that of progesterone (Shelton 1965). This apparent anomaly may be explained by a slightly longer half-life. The decline to basal plasma concentrations of progesterone and

Cronolone after withdrawal of impregnated sponges is rapid, but that of Cronolone lags by some 2 h (Gaston-Parry *et al.* 1988).

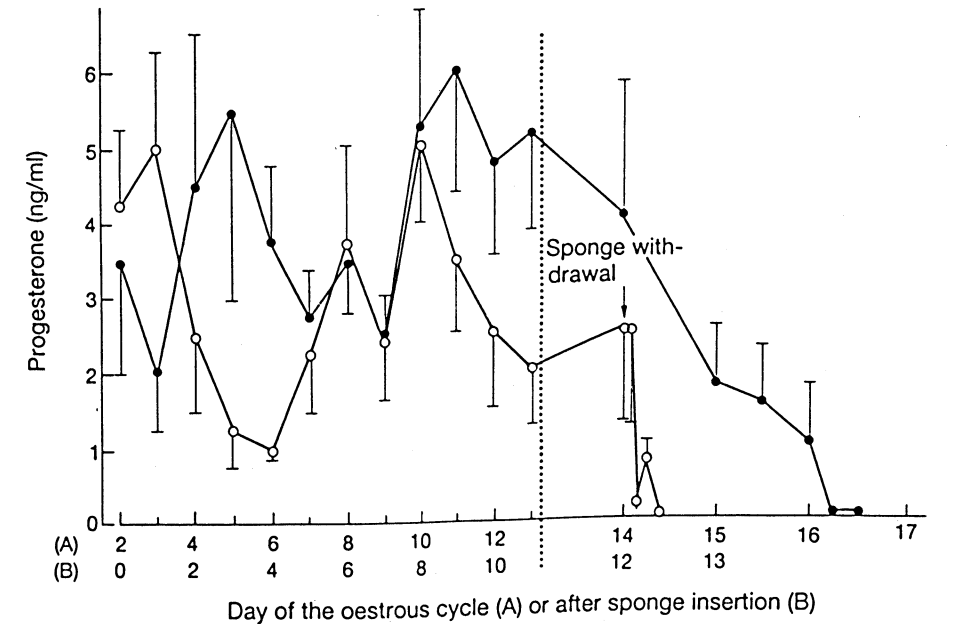


Fig. 3. Plasma progesterone concentrations (mean $\pm$ s.e.m.) during a normal oestrous cycle [A (●), $n = 8$] and during and after the insertion of progesterone impregnated intravaginal sponges [B, (○), $n = 16$], showing the marked difference in decline of the progesterone profiles. (Reproduced from Pearce and Robinson 1985, with permission.)

Table 1. Relationship between stage of oestrus when inseminated, LH release and numbers of spermatozoa in the cervix

Data from Pearce and Robinson (1985). Significance of differences in spermatozoa recovered: total ewes — early *v.* mid + late oestrus, $P < 0.05$; ewes with LH peak — 0.8 h *v.* remainder, $P < 0.02$

Stage of oestrus	Total ewes inseminated		Ewes with LH peak detected		
	No. of ewes	No. of sperm	LH to insemination (h)	No. of ewes	No. of sperm
Early (< 6 h)	16	1.5×10^6	< 0	2	1.2×10^6
Mid (6–14 h)	18	3.4×10^6	0–8	8	8.6×10^6
Late (> 14 h)	17	2.6×10^6	> 8	12	2.8×10^6
Totals and means	51	2.4×10^6	< 0 – > 8	22	5.7×10^6

Table 2. Plasma concentration of Cronolone during insertion of impregnated intravaginal sponges
Data are from Gaston-Parry *et al.* (1988). Significance of difference between doses: $P = 0.2$ (n.s.)

Cronolone dose (mg)	No. of ewes	Mean ($\pm$ s.e.m.) Cronolone plasma concn (ng/ml) for day of sponge insertion:						
		–1	1	3	5	7	9	11
40	11	0.3	4.3	3.6	1.6	1.2	1.3	1.5
		± 0.03	± 0.8	± 0.7	± 0.3	± 0.3	± 0.2	± 0.3
80	5	0.1	6.3	5.6	2.1	2.0	2.3	—
		± 0.04	± 0.6	± 0.4	± 0.3	± 0.3	± 0.3	—

Precision of Endocrinological Events Following the Use of Exogenous Progestagen

The close relationship between the times of LH release and ovulation in the cyclic ewe was demonstrated in the early 1970's, and Cumming *et al.* (1973) stressed this in relation to the time of insemination. The current interpretation was that an appropriate time of insemination was necessary for the transport of spermatozoa to the site of fertilization before ovulation. Our data, cited above (Pearce and Robinson 1985), show that this is not the only factor. The endocrinological environment, shortly after the release of LH, appears to be optimal for the establishment of a basic reservoir of spermatozoa in the cervix. Hence, the time of LH release, relative to that of progestagen withdrawal and the onset of oestrus, assumes paramount importance.

Earlier studies, from the laboratories of Goding (Cumming *et al.* 1970) and of Ortavant (Cognie and Pelletier 1976), had indicated considerable variability in these time relationships so, in 1981, an extensive and detailed re-investigation of these matters was initiated in collaboration with Dr R. J. Scaramuzzi and his associates at the Division of Animal Production, CSIRO, Prospect, N.S.W.

First of all, we established an effective and repeatable mating management practice using a 'saturation mating' technique in which the ewe to ram ratio never exceeded 3.5 to 1 (Robinson *et al.* 1987). This involves close scrutiny and regular drafting of mated ewes and it provides accurate data for time of onset of oestrus and the percentage of ewes in oestrus. Practically 100% of Border Leicester $\times$ Merino ewes treated in mid-anoestrus with Cronolone sponges and PMSG on withdrawal have mated, practically all by 48 h. Unmated ewes generally have been found to have been pregnant when treated. Time of first mating is highly predictable and follows a normal distribution, centred around 33 h, in ewes injected with 500 i.u. PMSG, commencing at 24 h and terminating by 51 h: 90% have mated by 42 h. This pattern is unrelated to time of day when treatment is terminated: there is no diurnal effect.

Secondly, we have determined accurately the time relationships between the various endocrinological and behavioural phenomena which follow treatment of anoestrous crossbred ewes with Cronolone sponges and PMSG on withdrawal. While there had been such studies in the past, all had looked at only part of the picture and broad conclusions were being drawn from fragmented data.

Serial bleeding for LH release of ewes joined at various times, and with accurately recorded times of first mating, revealed a remarkably close relationship between time of onset of oestrus and LH release (Fig. 4), confirming the tentative conclusion drawn from the earlier experiment of Pearce and Robinson (1985). The LH peak was at 4.5 ± 0.7 h after first mating, i.e. at a mean of 38 h after sponge withdrawal. The correlation coefficient between the times of onset of oestrus and release of LH was 0.93. There was no evidence of premature release of LH, as had been reported in a proportion of ewes by Cognie and Pelletier (1976). Two subsequent experiments, one using Fecundin-immunized and non-immunized ewes, confirmed these results. The use of Fecundin delayed the onset of oestrus: it delayed also the time of LH release to the same degree so that the time relationship between these two phenomena remained constant. Further, the time of ovulation relative to that of LH release remained constant at about the well-established interval of 24 h (T. J. Robinson, R. J. Scaramuzzi and Catherine A. Smith, unpublished data).

The difference between these data and those of Cumming *et al.* (1970, 1973) can be explained on the bases of method of detection of oestrus and our use of PMSG. There are no published data to account for the difference between our results and those of Cognie and Pelletier (1976), but Yves Cognie (personal communication) has advised us that premature release of LH is associated only with excessive stimulation with PMSG.

It is concluded, therefore, that the use of Cronolone sponge followed by PMSG treatment on withdrawal at normally recommended doses results in a predictable and precise series of endocrinological events. In practical terms, the range in times from sponge withdrawal and PMSG injection to the onset of oestrus in anoestrous crossbred ewes receiving standard treat-

ment of sponges containing 30 mg Cronolone for 12 days, followed by 500 i.u. PMSG at withdrawal, is 24–48 h. The release of LH starts some 3 h later, with the peak at 4–5 h. Ovulation occurs some 24 h after this peak, i.e. at about 52–76 h after sponge withdrawal, distributed normally about a mean of 64 h.

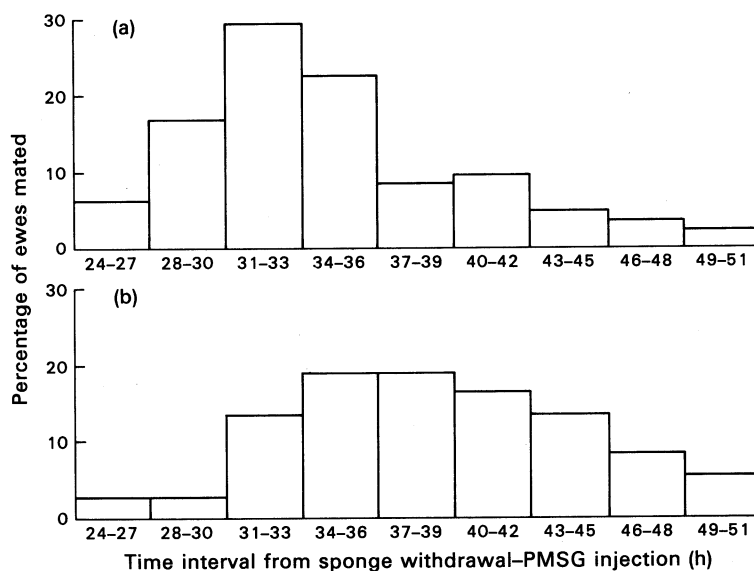


Fig. 4. Percentage distribution of time of mating (a) and of time of LH release (b) in relation to the time interval of sponge withdrawal and PMSG injection. In (a), ewes ($n = 82$) were joined at 24 h; in (b) data for all ewes with LH peak ($n = 39$) are given. The mean delay was 4.5 ± 0.7 h ($r = 0.93$). (Reproduced from Robinson *et al.* 1987, with permission.)

These limits of 24 h contrast with the much shorter limits of the time relationship between the onset of oestrus and LH release. It follows, therefore, that the factor limiting the precision of this system of controlled breeding probably is variation in time of follicular development, and associated oestrogen production, following the cessation of progestagen inhibition and initiation of gonadotrophin stimulation. In this connection, the quantitative temporal relationships between dose of oestradiol and the time of onset and duration of oestrus is well documented in spayed ewes (Robinson 1955; Fletcher and Lindsay 1971): an early onset of oestrus of long duration is associated with a high dose. A similar relationship exists in entire ewes, in which a high ovulation rate, and associated high oestrogen production, has a similar effect (Evans and Robinson 1980c). Unfortunately, our oestrogen assay has not been equal to the task of accurately measuring oestradiol- 17β in the relatively moderately stimulated animals of these experiments, so we are forced to fall back upon circumstantial evidence. It is postulated that there is a critical threshold of plasma oestradiol required to trigger two events, namely the manifestation of oestrous behaviour and the closely associated release of LH. It is interesting to speculate upon whether these two seemingly disparate phenomena are a *direct* result of steroid action or whether or not there is an intermediate link. Personally, I would be looking for such a link.

So, looking at these temporal relationships from a practical point of view, we have three options. First, we can use an intensive field-mating system, as described: while effective, it requires a large number of rams and very close observation and is labour intensive, but it is the preferred method for commercial operation. Second, we can use hand-mating, to rams of proven reproductive and genetic worth, at 36, 42 and 48 h: this probably is the best option for a normal stud operation. Third, if artificial insemination is to be used, fixed-time insemination, as developed by the French, with two inseminations at 48 and 56 h after sponge withdrawal

and PMSG treatment is probably the best system for an advanced stud operation. Our data, which show the importance of time of insemination relative to that of LH release, suggest that the times could well be advanced by 6 h. Further, the advent of intra-uterine insemination by endoscopy may well alter the picture as far as stud breeding is concerned.

Another point to be borne in mind is that the statistical precision of this technique can pose problems because of the paramount importance of time and efficiency of insemination, whether natural or artificial. The conventional method of artificial insemination involves the daily mustering of the flock and the drafting off of oestrous ewes for insemination. In a situation of natural oestrus, the time of onset of oestrus, and of LH release, will have been at random during the past 24 h, these phenomena following a normal distribution centred around 12 and 7 h before drafting. The chances of optimal sperm transport will follow this normal distribution and some two-thirds of ewes could be expected to fall within the optimal time category. By contrast, ewes in which oestrus has been synchronized with progestagen sponges and PMSG treatment and which are drafted daily for insemination, would not have such a random distribution.

Given the normal management situation in Australia, ewes from which sponges were withdrawn at 0800–0900 h on Monday, for example, would be mustered on Tuesday morning. Oestrous activity would be just starting but, in all probability, none would be drafted for insemination. On Tuesday afternoon they may be drafted, but it is unlikely that any would be inseminated until the following day. The following morning, Wednesday, they would be mustered again. Oestrous ewes, of which there would be many, would be drafted off and would be inseminated within a few hours, that is at between 48 and 52 h after sponge withdrawal, or at a mean of 9–14 h after the LH peak. This is marginally too late. The following morning, the remaining 'oestrous' ewes would be drafted and inseminated: in their case, far too late. The importance of these time relationships cannot be overemphasized: the entire success or failure of a controlled-breeding enterprise depends upon it.

Effects of Equine Serum Gonadotrophin (PMSG)

The difficulty with PMSG is that it is not a clearly defined hormone. Its structure and physiological activity have been debated ever since its discovery in the late 1920's (Cole and

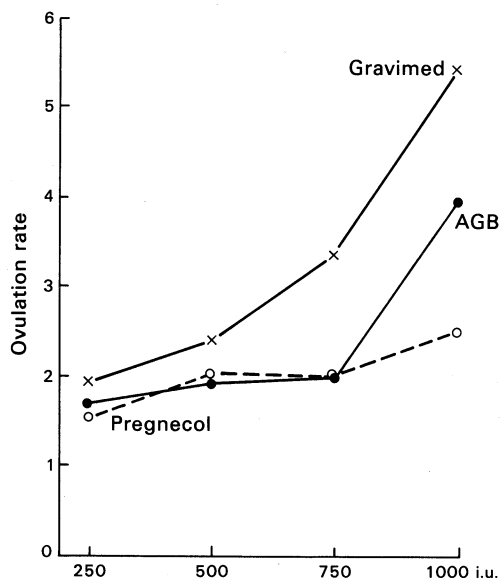


Fig. 5. Differences in ovulation rates of ewes treated with three batches of PMSG all of which were standardized in the same laboratory by the same standard mouse uterine weight test. (Reproduced from Robinson and Scaramuzzi 1986, with permission.)

Hart 1930). We compared the response of the anoestrous ewe to three preparations, each of which was standardized by Dr Angus Gidley-Baird, Department of Veterinary Physiology, University of Sydney, using a standard mouse uterine weight test. The responses differed widely,

as shown in Fig. 5. All would induce ovulation but only two would induce superovulation, one much better than the other. Such differences make generalizations difficult.

With that qualification, PMSG has two major characteristics. First of all, it will reliably induce ovulation in anoestrous sheep and goats and, for most preparations, increasing numbers of ovulations with increasing doses. Secondly, it is steroidogenic. Production of oestradiol increases linearly with increasing numbers of ovulations but there is also a residual effect not accountable by the number of developing follicles (Evans and Robinson 1980c).

The importance of these quantitative aspects of oestrogen and/or progesterone influence in relation to the establishment and maintenance of pregnancy cannot be overemphasized. French workers were the first to recognize the importance of the physiological state in controlled-breeding programs. Essentially, what they did was to apply increasing doses of progestagen and of PMSG to increasingly difficult physiological states, ranging from the cyclic condition to that of lactation in deep anoestrus (Thimonier and Cognie 1977). Our experience conforms to this concept. By the use of endoscopy of 305 crossbred ewes between days 18 and 20 after mating following Cronolone sponge and PMSG treatment, we have data relating dose of PMSG and consequent ovulation rate to ewes impregnated (i.e. with one or more functional corpora lutea at endoscopy), ewes lambled and lambs born. At endoscopy, the numbers of active, regressing and regressed corpora lutea (corpora albicans) were recorded. As these were anoestrous (acyclic) ewes no difficulty was experienced in differentiating between these categories or with identifying the few recent ovulations of the next cycle. From these data, we have been able to relate the ovulation rate to the chances of pregnancy at various stages of anoestrus and to estimate the losses of ova from ovulation to lambs born (Robinson *et al.* 1987).

A high chance of a ewe becoming impregnated depends upon a high ovulation rate. This is illustrated in a number of ways. Table 3 shows the mean ovulation rate of ewes which become impregnated to be 50% higher than that of ewes in which pregnancy is not established.

Table 3. Relationship between mean numbers of ovulations and pregnancies established in 200 crossbred ewes in anoestrus

Data from Robinson *et al.* (1987). ***Difference significant at $P < 0.001$

Method of pregnancy diagnosis on day 19	No. of ewes		Mean No. of ovulations	
	Pregnant	Non-pregnant	Pregnant ewes	Non-pregnant ewes
Endoscopy	125 (62.5%)	75 (37.5%)	2.7***	1.8
Plasma progesterone	123 (61.5%)	77 (38.5%)		

Table 4. Relationship between numbers of ova shed, ewes impregnated, ewes lambled, lambs born and ova lost
Data from Robinson, *et al.* (1987). Significance of all linear series of percentages: $P < 0.001$

Ova shed/ ewe	Total No. of ewes	Data for ewes subjected to endoscopy			No. of lambs born	No. of lambs per ewe	Ova lost (%)
		No. of Ovula- tions	No. impreg- nated	No. which lambled			
1	134	134	80 (60%)	71 (53%)	71 (53%)	1.0	(47%)
2	97	194	66 (68%)	58 (60%)	78 (80%)	1.3	(60%)
≥ 3	74	420	62 (82%)	56 (76%)	86 (116%)	1.5	(72%)
Total	305	748	208 (68%)	185 (61%)	235 (77%)	1.3	(63%)

Table 4 shows that increasing the numbers of ovulations from one to three results in a linear increase in the percentage of ewes which are impregnated and which lamb, and in the number of lambs born per ewe. The importance of this in relation to the chances of impregnation increases as the physiological condition becomes more difficult and additional data, yet to be fully analysed, show this to be particularly important in early lactation: in our experience,

only 45% of ewes in early lactation early in seasonal anoestrus have been impregnated even when three ova have been shed.

It is clear that effective controlled breeding requires a high ovulation rate — ideally every ewe should shed two or three ova. Even this will not result in acceptable percentages of impregnations in ewes in early lactation. Recognition of this led to the abandonment of the '49 day' breeding system investigated at Nouzilly a decade ago (Thimonier and Cognie 1977). The reasons for failure of ewes to conceive early in lactation was a topic studied two decades ago by Fletcher (1968) but, despite its importance, it has received relatively little attention. However, a high ovulation rate in mid to late lactation can lead to regular impregnation percentages of 65–75% and higher. Corresponding percentages for ewes with but one ovulation are unacceptably low at 60% or less. As the breeding season approaches and lambs are weaned, the importance of ovulation rate decreases.

Once pregnancy is established, subsequent losses are relatively small. Table 4 shows the overall discrepancy between the percentage of ewes impregnated and ewes lambing to be 7%, representing a 10% loss of litters by impregnated ewes. Further, there is a linear decline in the percentage of litters lost with increasing numbers of ovulations.

There is a common fear that a high ovulation rate will lead to many triplets and quadruplets. In general, the pattern of early embryonic mortality described by Robinson (1951) looks after this: litter sizes are reduced early in pregnancy to what the uterus can support and, while they do occur, litters larger than three are not common. In the current program, quadruplets have been rare and triplets scarce, and the occasional large litter must be accepted as one of the hazards of a controlled-breeding program. Twins are common and, unless one is in a position to cope with these, one should not be involved in such a program.

At one time I believed that the role of a high ovulation rate was basically due to the statistics of the situation: 40% of ova were doomed to perish (Brambell 1948) and therefore, the more eggs shed, the greater the chance that one would survive and establish a viable pregnancy. Now I am not so sure. A high ovulation rate is associated with a high level of steroidogenesis. A high plasma concentration of oestrogen can be expected to result in a high intensity of oestrous behaviour and of vaginal and uterine stimulation, followed by high luteal activity. Recent studies at the Department of Animal Husbandry, University of Sydney, Camden, N.S.W. (e.g. Miller and Moore 1983, Moore *et al.* 1983), on the biochemical changes in the endometrium of spayed ewes following various steroidal regimes and the consequent effect on the establishment of pregnancy, have shown the importance of these relationships. So, my current feeling is that a high degree of steroidogenesis may be as important as the actual number of ova shed: in short, reluctant ovulations coaxed by minimal stimulation are unlikely to result in a high proportion of ewes impregnated. Moderate overstimulation is better than understimulation, leaving the natural regulatory mechanisms of embryonic mortality to reduce litter sizes to within the normal range. Gross overstimulation is counter-productive, resulting in accelerated transport of ova to the uterus and failure of fertilization (Robinson 1951).

Conclusions

The fundamental questions, why does the Australian Merino ewe differ from its British counterparts in its seasonal cyclic ovarian pattern and why does the ram differ in its behavioural response to endocrinological changes induced by the photoperiodic environment, remain enigmatic. As discussed above, there were no measurable breed differences in the endocrinological patterns studied. Further, the patterns of pulsatile LH release were similar in all rams, and appeared unrelated to photoperiod. There were cyclic differences in LH patterns in ewes but these were related to the stage of the oestrous cycle and, as for the rams, no functional role on seasonality could be ascribed to light-controlled changes in LH patterns in any breed (Poulton and Robinson 1987).

Despite the relative freedom from constraint of breeding activity of the Merino, there is

still a considerable residual effect of photoperiod. In the rams, this is particularly evident in the pattern of scrotal sac volume and associated testosterone production but, even when both are at a minimum, the mating drive is never lost. In the ewes, a proportion can be expected to be entrained to photoperiod — in our case 1 in 4 — while the others are not. It follows that, while the response of the pineal gland to photoperiod and the consequent pattern of release of melatonin may well be the unifying factor involved in the response of the sheep to photoperiod, this regulatory role is not absolute. Hence, results obtained in highly seasonal breeds will need careful interpretation in relation to those in less seasonal breeds, of which the Merino may well be an extreme example. Modifying factors will be found, of which one — the so-called 'ram effect' — has been identified (Schinckel 1954). The nature and importance of these will vary between breeds.

The only conclusion that can be drawn, imprecise though it may be, is that the Merino sheep, over a long period of time, has not been subjected to the same rigorous natural selection pressure for a restricted breeding season as have other European breeds and so is not so firmly entrained to the photoperiodic environment. Artificial selection pressure for autumn lambing in Australia probably has increased this, so resulting in a considerable freedom from such constraint and an ability to respond to other environmental cues.

The second issue raised, namely the requirements for the establishment of adequate reservoirs of spermatozoa in the cervix, is more noteworthy for its negative than its positive aspects. It has been established that insemination shortly after the LH peak is necessary, but there is no indication as to why. The situation has been defined and is open to an immediate and intensive positive study.

The third issue is that of the time relationships between the withdrawal of a progesterone — progestagen regime, whether by natural luteolysis or by artificial withdrawal, and ensuing follicular development and the associated onset of oestrus, LH release and ovulation. Our data indicate that the factor limiting the precision of these events is variation in the time to attain a plasma concentration of oestrogen sufficient to induce the surge of LH. An oestrogen assay more sensitive than any readily available today is needed to resolve this. This is important, because upon it depends our ability to improve further the precision of control of breeding.

From a practical point of view, the desirable degree of precision depends largely upon the system of insemination to be used. For artificial insemination on a small scale, when all ewes can be inseminated over a short period, preferably on a fixed time basis, extreme precision is needed. Less precise control may well be an advantage for hand-mating. If natural mating in the field is to be used, precision is much less important. The critical issue is mating pressure. A seeming excess of rams must be joined and the whole mating process must be managed carefully so that ewes are withdrawn shortly after mating and put to 'back up' rams. The importance of this cannot be overemphasized. Mating management has been the most neglected area in the exploitation of controlled breeding.

This brings us to the fourth issue, namely the manifold effects of PMSG. In order to exploit an effective system of mating or artificial insemination, ewes must be prepared to maximum advantage. Apart from use of such obvious management practices as good nutrition and freedom from disease, ewes must be induced to have a high ovulation rate and associated level of steroidogenesis. There is a good deal of user opposition to this because of the fear of multiple births. This is a real, but overemphasized, hazard and one which must be accepted in any controlled breeding program. Some PMSG preparations induce only a mild degree of superovulation. A detailed investigation of the reasons for differences between preparations should be given a high priority, because therein may lie the solution to the problem of overstimulation.

The current technology for controlled breeding has stood the test of time, despite its acknowledged limitations: it will continue to be used in the foreseeable future. The intravaginal route for the administration of progestagens is now well accepted and, although new vehicles for application are being developed, these will need to be subjected to the same rigorous examination as has been described here. The existing sponge tampon may be superseded, but this

will not resolve the question of rapid collapse of the progestagen regime on withdrawal. Further, a recognition of the necessity for a high degree of ovarian stimulation, with an associated degree of superovulation, is essential: this is particularly important in the more intransigent physiological states of deep anoestrus and lactation. In early lactation (i.e. within 2 months of lambing), even with a high degree of stimulation accompanied by a high ovulation rate, it is not possible to achieve an acceptable lambing rate approaching 50%. The biology of the lactating ewe and the factors — environmental, physical, physiological and endocrinological — which render it so intransigent to the re-establishment of an early pregnancy is the most important area for investigation over the next five years.

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