Improving the success of sheep artificial insemination programs

A handbook for practitioners

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Summary

Research outlined in this document aimed to improve the success of fixed-time AI programs in sheep. The underlying hypothesis was that the method of synchrony of oestrus, namely the use of the progesterone pessary, adversely affects follicle development resulting in poor synchrony and compromised oocyte quality. This hypothesis is supported by the findings of the study. It is concluded that emergence of the ovulatory follicle during pessary treatment is controlled by regression of the corpus luteum of the previous cycle. As a result, at the time of pessary removal, ovulatory follicles vary widely in age and it is this variability that influences the functionality of the ovulatory follicle and, subsequently, timing of oestrus and the ability of the ewe to conceive. In addition, flock management factors, most noticeably long and short term nutrition, can impact synchrony of oestrus with consequence for AI outcomes. Attempts to develop improved protocols have centred on controlling the time of emergence of the ovulatory follicle. These protocols have increased the potential lambing rate (fetuses/100 ewes inseminated) by up to 35% in association

with improvements in synchrony of oestrus. Research is ongoing with a need to evaluate these new protocols in Spring as well as in commercial flocks. These studies are planned for the 2021/22 breeding season.

Background

This document outlines the findings of research conducted to improve the success of sheep AI programs. The project, funded by Australian Wool Innovations Ltd (AWI), was titled "Improving the success of sheep AI programs". It was conducted between 2018 – 2021 at Turretfield Research Centre, South Australian Research and Development Institute (SARDI) with support from the South Australian Stud Merino Breeders Association (SASMBA) and the Australian Association of Stud Merino Breeders Ltd.

Anecdotal evidence accumulated over several decades indicates that the success of AI programs has remained highly variable with little or no improvement. The SASBMA conducted a state-wide survey of the 2011 and 2012 seasons to quantify success rates and to identify causes of failure. Of the 32 respondents involving 54 flocks, 12 reported pregnancy rates below 50% in at least one of the two years including six who reported rates below 35%. Information indicated that poor synchrony of oestrus was the most likely cause of these results. These data indicated that there was a need to re-evaluate the methodology of synchronisation (largely unchanged for 50 years) and to develop new strategies to improve AI success rates.

This project used trans-rectal ultrasonography to examine ovarian activity during the period of pessary treatment. From these data, new information on the relationships between follicle growth patterns, luteal regression and reproductive outcomes have been obtained. This information facilitated the development of new strategies to improve synchrony of oestrus including changes to treatment protocols as well as flock management.

Variations in patterns of oestrus

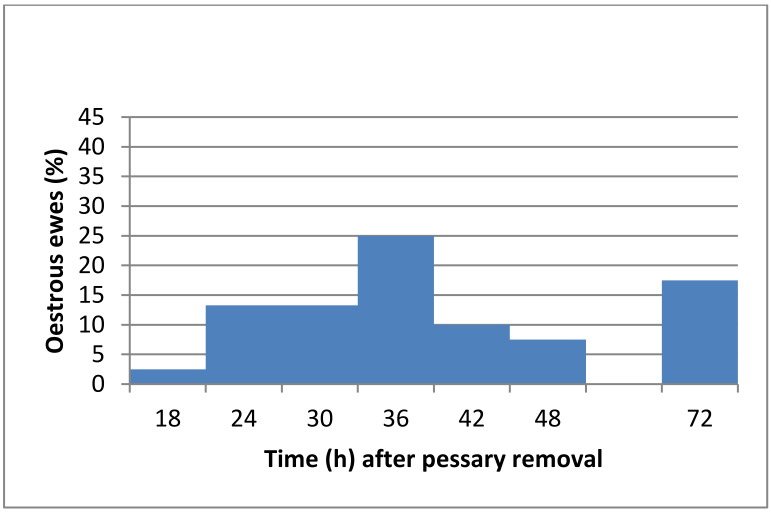
Patterns of oestrous synchrony range from being very good to being very poor (Figure 1a - e) raising the question of what is a normal pattern? The normality of oestrus can be assessed using the following criteria:

- Time of first oestrus. The best opportunity to gauge normality occurs 24h after pessary removal. Generally, a minimum of 10 – 20% of ewes should be in oestrus although this figure can be as high as 40 - 50%. The absence of oestrous ewes at this time is indicative of a delayed synchrony.

- The percentage of the flock not detected in oestrus at the commencement of insemination (42h after pessary removal). Ewes that cycle after this time have a reduced chance of conceiving.
- The percentage of the flock that fails to come into oestrus (usually 10 – 20%). Ewes in this category include those with clinical conditions (e.g. *Hydrops uteri*). Overall, 30 – 40% of ewes that fail to be detected in oestrus conceive to AI.

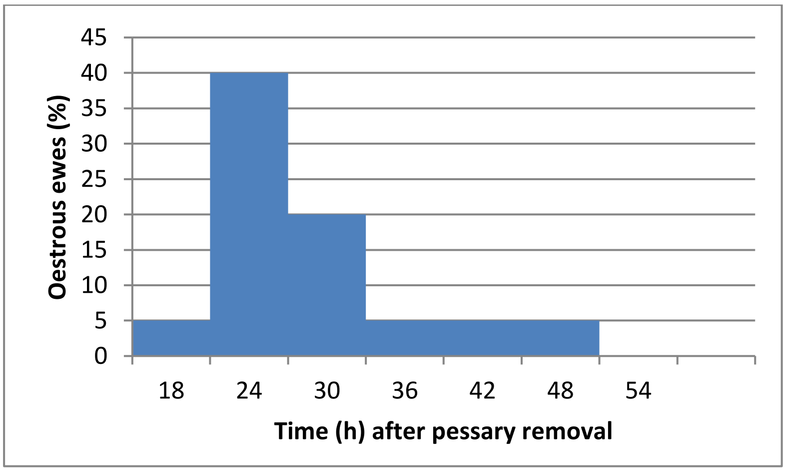
Figure 1. Examples of timing of onset of oestrus following pessary removal. These charts plot the percentage of new ewes in oestrus (using teasers with harnesses and crayons) at 6-h intervals after pessary removal. Observations were conducted up to 48h with a final observation at 72h.

a. Flock 1, 2020 (CIDRs; ewes in oestrus = 87.5%)



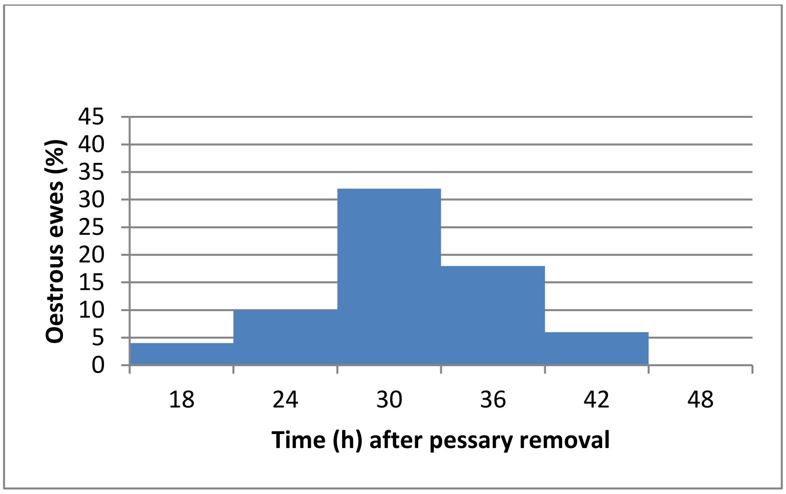
- 15.0% in oestrus at 24h
- 12.5% not in oestrus
- 17.5% in oestrus after 48h

b. Flock 2, 2018 (CIDRs; ewes in oestrus = 80.0%)



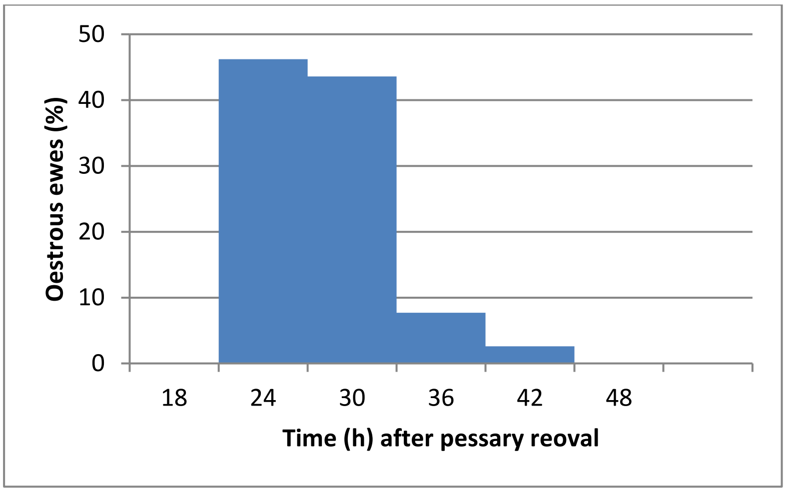
- 45.0% in oestrus at 24h
- 20.0% not in oestrus
- 0.0% in oestrus after 48h

c. Flock 3, 2018 (CIDRs; ewes in oestrus = 70.0%)



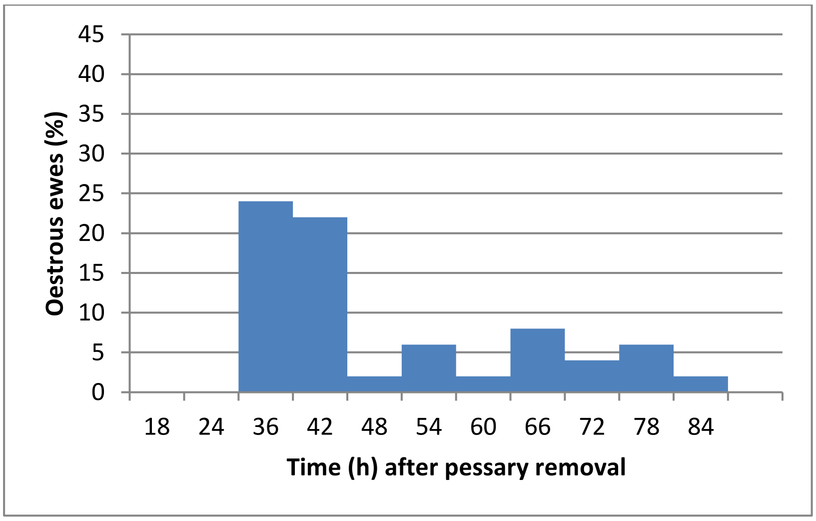
- 14.0% in oestrus at 24h
- 30.0% not in oestrus
- 0.0% in oestrus after 42h

d. Flock 4 (historical; sponges)



- 46.0% in oestrus at 24h
- 2.5% not in oestrus
- 0.0% in oestrus after 48h

e. Flock 5 (historical; sponges)



- 0.0% in oestrus at 24h
- 24.0% not in oestrus
- 30.0% in oestrus after 48h

Variations in these patterns have ramifications for AI programs particularly in relation to the timing of insemination. Data on Flock 4 and Flock 5, although historical, are presented to indicate how good (Flock 4) and bad (Flock 5) the synchrony can be.

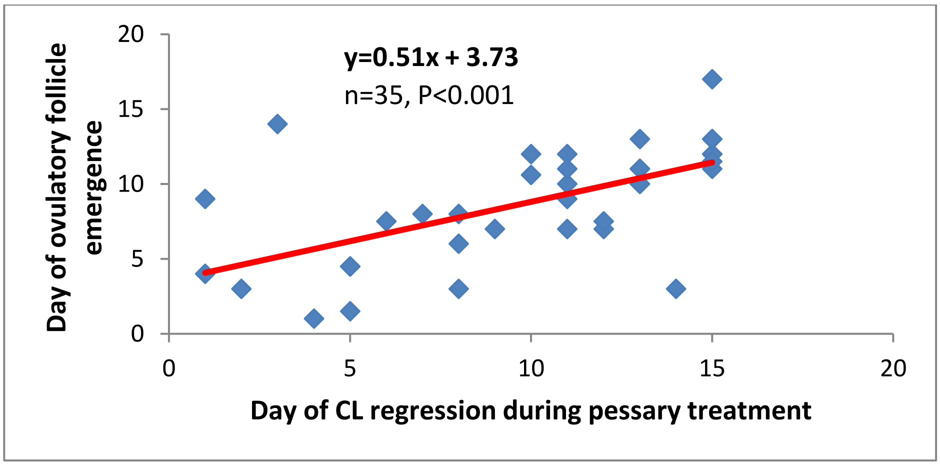
Follicle growth patterns during pessary treatment

The underlying hypothesis of this study was that pessary treatment adversely affects follicle quality resulting in poor synchrony of oestrus and compromised oocyte quality. To study follicle development, trans-rectal ultrasonography was used on a daily basis in ewes treated with pessaries and in ewes that were cycling naturally. From these observations it was possible to develop follicle maps (see Attachment) that provide information on follicle numbers, follicle size, day of emergence of the ovulatory follicle and the day of regression of the corpus luteum (CL).

Observations were made at three times of the year (Spring, Autumn and the Spring equinox) with two replicates conducted on each occasion. The following is a brief outline of some of the findings.

- In treated ewes, there was a significant ($P<0.001$) relationship between the time of CL regression and the day of emergence of the ovulatory follicle (Figure 2). The age of the ovulatory follicle, at pessary removal, ranged from 1 to 14 days or more.

Figure 2. Relationship between the day of CL regression during pessary treatment and the day of emergence of the ovulatory follicle.



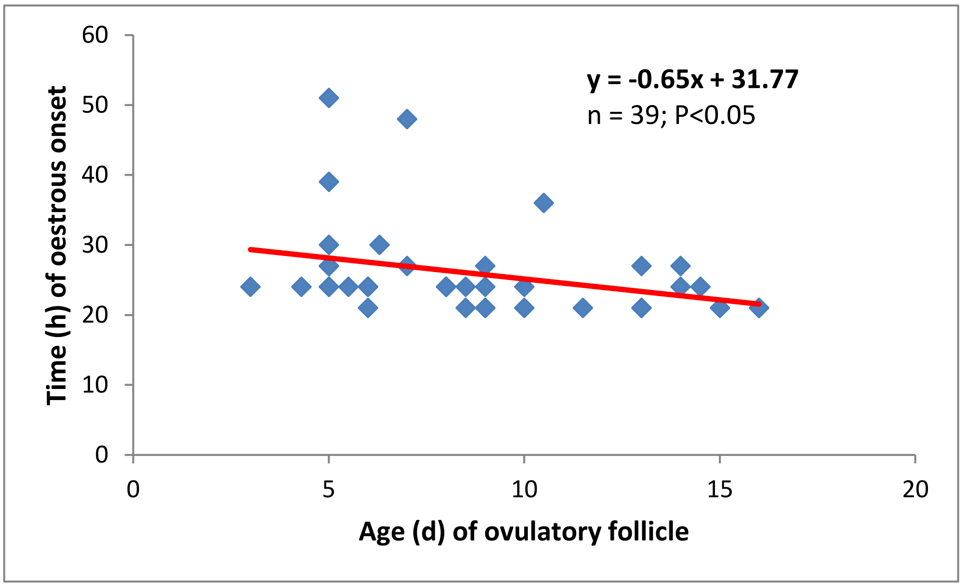
- The inter-ovulatory interval in ewes with multiple ovulations was more variable in ewes treated with pessaries (range 1-13 days) than in naturally cycling ewes (range 1-5 days) although mean values did not differ significantly (2.1±0.66 days versus 1.4±0.24 days respectively).

- In Autumn, treated ewes had fewer follicles than naturally cycling ewes presumably due to the early formation of dominant follicles. The converse applied in Spring when treated ewes had more follicles than naturally cycling ewes.
- Irrespective of differences in the total number of follicles, treated ewes had more large follicles late in the treatment period compared with naturally cycling ewes. Additionally, these follicles were larger in the former group.
- The incidence of luteinised follicles was higher in treated ewes than in naturally cycling ewes.
    - These follicles usually appeared within 2-3 days of pessary insertion.
    - They were more common in Spring than Autumn.
    - Morphologically, they developed and regressed in a manner typical of a normal CL.
    - Progesterone profiles from these luteinised follicles are expected to be similar to those produced by normal CLs (assays being conducted). The significance of this extra progesterone is not known but it may "prime" the ewe's cycle in Spring and this may be beneficial.
- Regression of CLs followed by re-ovulation after the induced ovulation occurred in some treated ewes but not in naturally cycling ewes (7.7% versus 0.0%).
- PMSG treatment did not significantly increase the size of ovulatory follicles compared with those obtained in naturally cycling ewes in either Spring or Autumn.

Association between the age of ovulatory follicles and AI outcomes

A significant negative correlation was found between the age of the ovulatory follicle and timing of oestrus (Figure 3). Ewes with older follicles came into oestrus earlier and with less variability than ewes with younger follicles. It is likely that these differences resulted from differences in oestrogen production (assays being conducted). Overall, approximately 42% of the variability in the timing of oestrus was found to be due to the age of the ovulatory follicle.

Figure 3. Relationship between age of the ovulatory follicle and time of onset of oestrus following pessary removal.



A significant relationship was also found between the day of CL regression (synonymous with follicle age) and pregnancy rate (Table 1). Ewes in which CL regression occurred between day ≥7-9 were significantly (P<0.05) more fertile than ewes in which regression occurred between either days 1-6 or days ≥10. The latter group (i.e. those with young ovulatory follicles) were the least fertile. There was no comparable relationship for litter size.

Table 1. AI outcomes in ewes in which CL regression occurred at different times during pessary treatment.

Day of CL regression	Pregnant ewes (%)	Litter size (fetuses of ewe pregnant)
1 - 6	76.6 (36/47)[b]	1.53 (55/36)[a]
≥7 - 9	84.0 (42/50)[a]	1.33 (56/42)[a]
≥10	69.9 (121/173)[b]	1.38 (167/121)[a]

Values within columns with different superscripts differ significantly (P<0.05). Ewes were inseminated with chilled semen.

In conclusion, pessary treatment results in the formation of three distinct groups of ewes:

- Those with aged ovulatory follicles. These emerge between days 1 – 6 and are likely to be highly oestrogenic. These ewes come into oestrus early and with minimal variability and they are of moderate fertility.
- Those with ovulatory follicles of intermediate age. These follicles emerge between days ≥7 – 9 and are expected to be moderately oestrogenic. These ewes come into oestrus in between the other two groups and with minimal variability. They are the most fertile ewes.
- Those with young ovulatory follicles. These follicles emerge after day ≥10 and are expected to be poorly oestrogenic. These ewes come into oestrus later and with greater variability and they are the least fertile of the three groups.

In addition to these effects, pessary treatment also extends the life of the CL (estimated to be up to 5 – 6 days) thus delaying luteal regression and the emergence of the ovulatory follicle. This delay results in a disproportionately larger number of ewes in the least fertile group (see Table 1) thus contributing further to the adverse effects of pessary treatment.

Strategies to improve AI success rates – changes to the treatment protocol

Two broad strategies to improve the treatment protocol have emerged from these studies. They are (1) controlling the time of ovulatory follicle emergence and (2) improving the quality of follicles, particularly those that emerge late in the pessary period.

1. **Controlling the time of emergence of the ovulatory follicle**

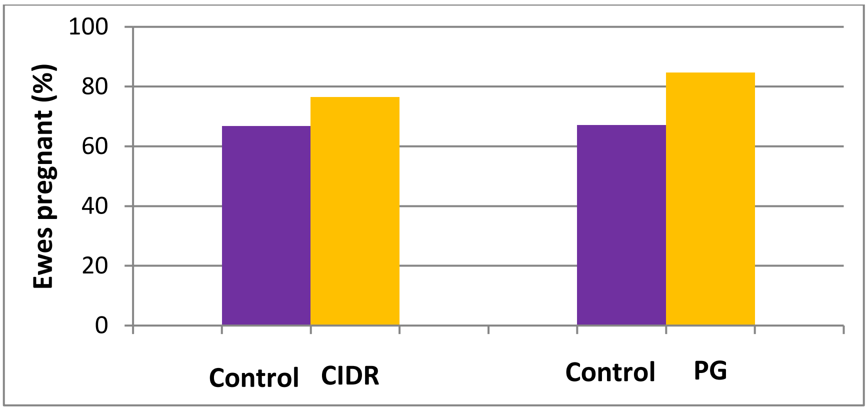
   Controlling luteal regression such that ovulatory follicles emerge ≥7 – 9 days of the pessary period should improve both synchrony of oestrus and pregnancy rates. Options examined were pre-treatment with either CIDRs or PGF2α, treatment with GnRH or exposure to teasers at the appropriate time (the latter two are specific to Spring).

   a. **Pre-treatment with CIDRs or PGF2α**

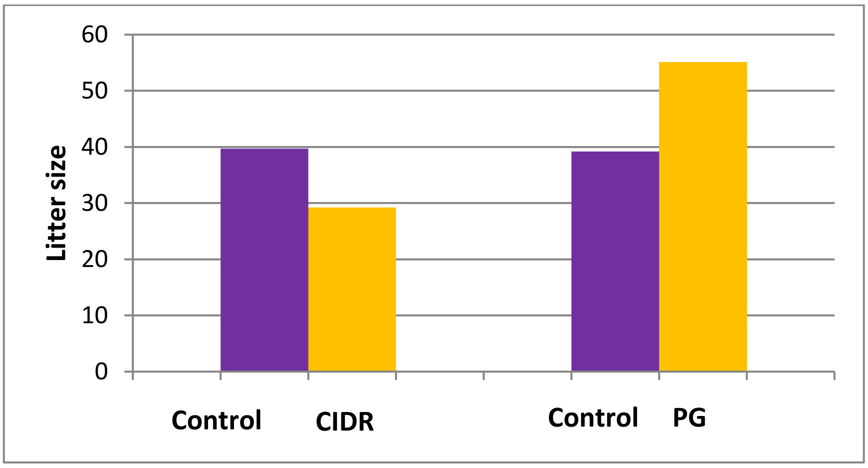
      In these studies, the first CIDRs were inserted 21 days before the second CIDRs whilst PGF2α treatment occurred 27 days before CIDR insertion. Details of each protocol are provided in Appendix Tables 2 and 3. Both pre-treatments increased pregnancy rates (Figure 4a) but only the PG pre-treatment increased litter size (Figure 4b). Overall, the PG pre-treatment significantly increased the number of fetuses/100 ewes by 35% (Figure 4c)

Figure 4. The effect of pre-treatment with either CIDRs or PGF2α on AI outcomes following AI with frozen-thawed semen.

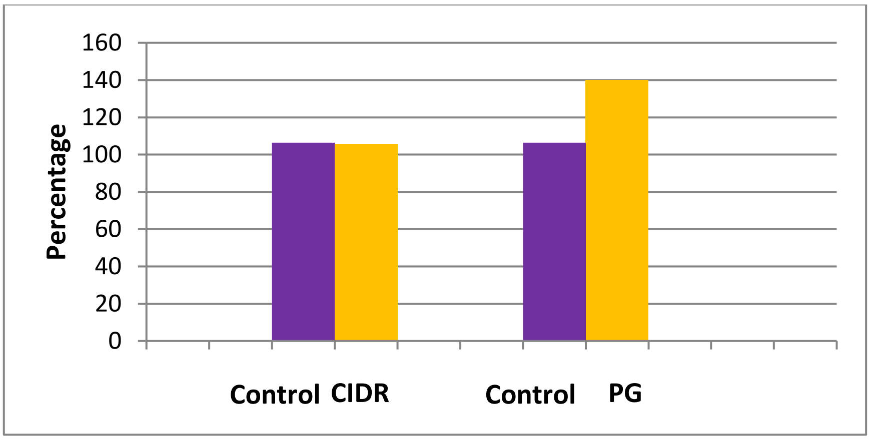
(a) Pregnancy rate



(b) Litter size (fetuses per 100 ewes pregnant)



(c) Fetuses/ewes inseminated



With both treatments there were indications of an improved synchrony but the changes were not significant.

Earlier PGF2α treatments (given either 12 days before pessary removal, 12 days before and six after pessary removal or six days after pessary removal) failed to improve any parameter. In fact, treatments involving administration on day 6 produced a delayed onset of oestrus and/or significantly lower pregnancy rates. It is possible that treatment at this time either failed to control the time of follicle emergence and/or produced an adverse side effect.

b. Exposure to teasers or treatment with GnRH in Spring

Appropriate timing of the induced ovulation using either of these options provides a potential means of controlling the emergence of the ovulatory follicle. Treatments examined were exposure to teasers eight days before pessary insertion and treatment with GnRH either at pessary insertion or six days later.

Exposure to teasers was more effective in inducing ovulation (100.0%) than was either GnRH treatment (68.0% and 70.6%). However, there were no differences in the timing of oestrus, the number of ewes in oestrus (88.2 – 89.8%), pregnancy rate (59.0 - 67.0%) or litter size (1.25 – 1.36). Interestingly, in the Control group, significantly more previously anoestrous ewes became pregnant (81.0%) compared with cycling ewes (43.4%) and significantly more ewes with fresh CLs at pessary insertion became pregnant (69.2%) compared with ewes with aged CL (10.0%).

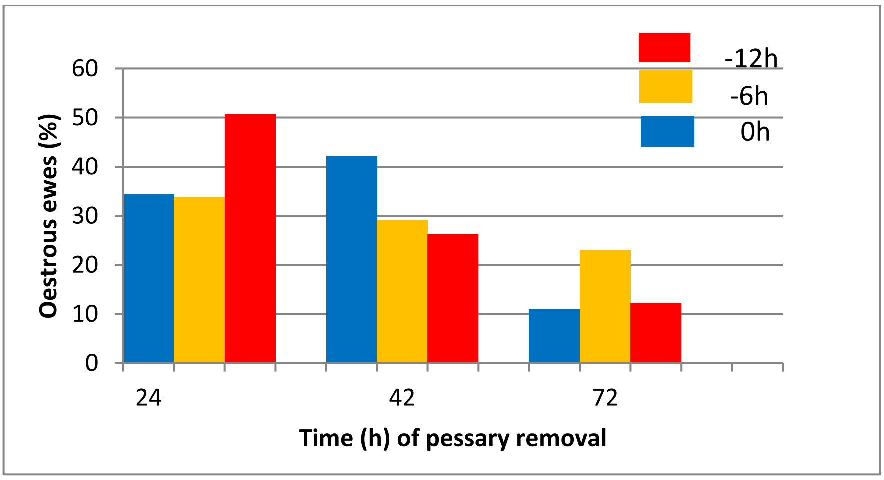
2. Treatment with PMSG before pessary removal

Early treatment with PMSG has the potential to improve the performance of ewes with younger ovulatory follicles. In the first of two experiments, the effects of PMSG treatment at -48h, -24h and 0h (relative to pessary removal) were examined. Synchrony of oestrus was significantly improved but the total number of ewes in oestrus declined from 87.5% in the 0h group to 78.0% and 72.5% in the -24h and -48h groups respectively. Furthermore, neither pregnancy rate (61.6 – 64.9%), litter size (1.46 – 1.65) nor ovulation rate (1.86 – 2.27) were significantly affected. It is likely that the benefits of an improved synchrony were lost because of the increase in the number of ewes failing to come into oestrus.

A second experiment was conducted to examine the effects of PMSG treatment at -12h, -6h and 0h. Relevant findings were:

- Treatment at -12h but not -6h significantly improved synchrony of oestrus (Figure 5).
- The total number of ewes in oestrus was not affected by treatment (86.2 – 89.2%).
- Treatment at -12h induced overt sexual behaviour (the mounting of teasers and other ewes) indicating an increase in the intensity of oestrus.
- Both pregnancy rate and litter size were increased by treatment at -12h compared with the other groups. These increases resulted in a 17% increase in the number of fetuses/100 ewes inseminated (Figures 6a,b).

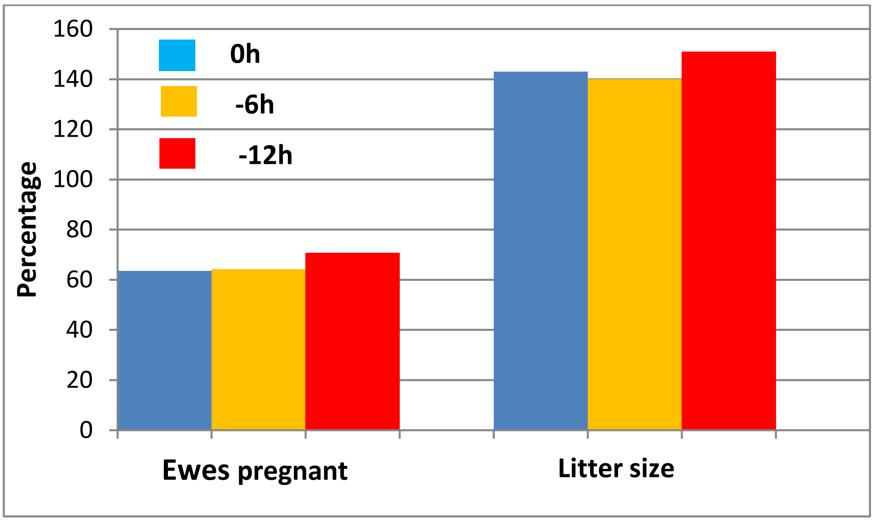
Figure 5. Effect of PMSG treatment at 0h, -6h and -12h relative to pessary removal (0h) on time of oestrus (ewes were observed daily).



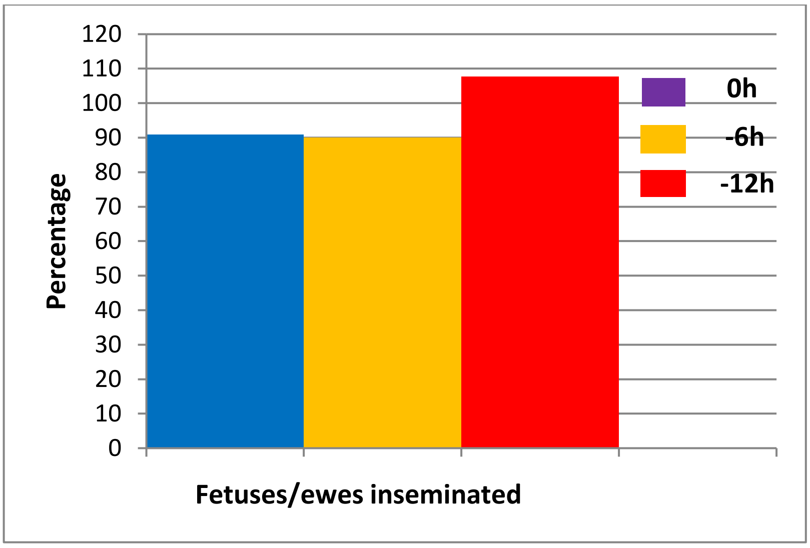
In conclusion, PMSG treatment at -12h markedly improved synchrony of oestrus without adversely affecting the percentage of oestrous ewes and resulted in a worthwhile increase in the potential lambing rate.

Figure 6. Effect of PMSG treatment at 0h, -6h and -12h relative to pessary removal (0h) on AI outcomes (ewes were inseminated at 43h with frozen-thawed semen).

(a) Ewes pregnant and litter size



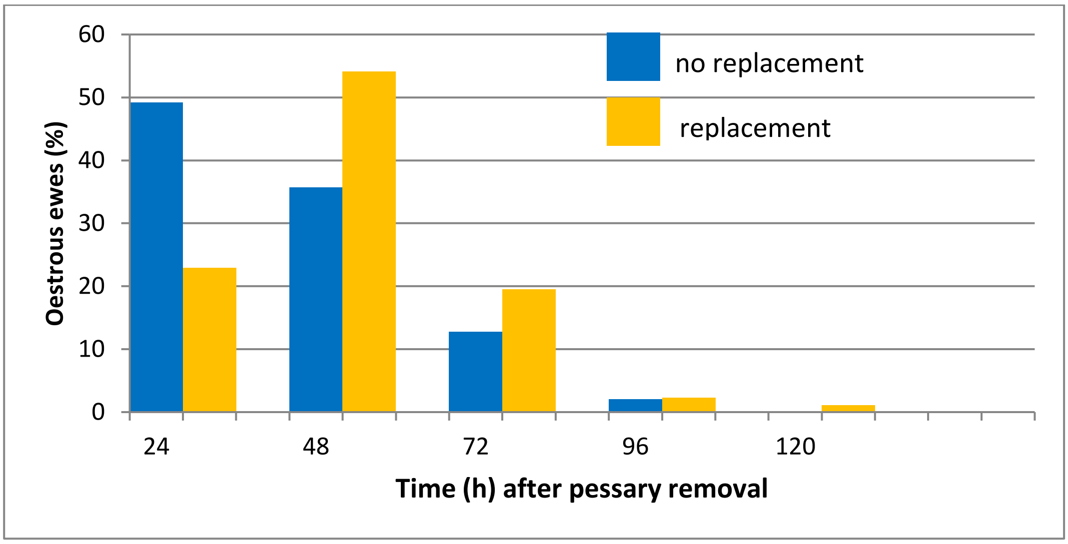
(b) Fetuses/ewes inseminated



3. Pessary replacement on day 9

Pessary replacement addresses the issue of progesterone levels during the treatment period falling below normal luteal levels. This situation is exacerbated under conditions of high nutrition given the inverse relationship that exists between circulating levels and dietary intake. Not surprisingly, pessary replacement produced a significant delay in the pattern of oestrus (Figure 7). Further research is required to determine the significance of this delay but it may be useful in flocks with a history of poor synchrony given the potential for associated improvements in follicle quality.

Figure 7. Effect of pessary replacement (day 9) on the timing of oestrus.



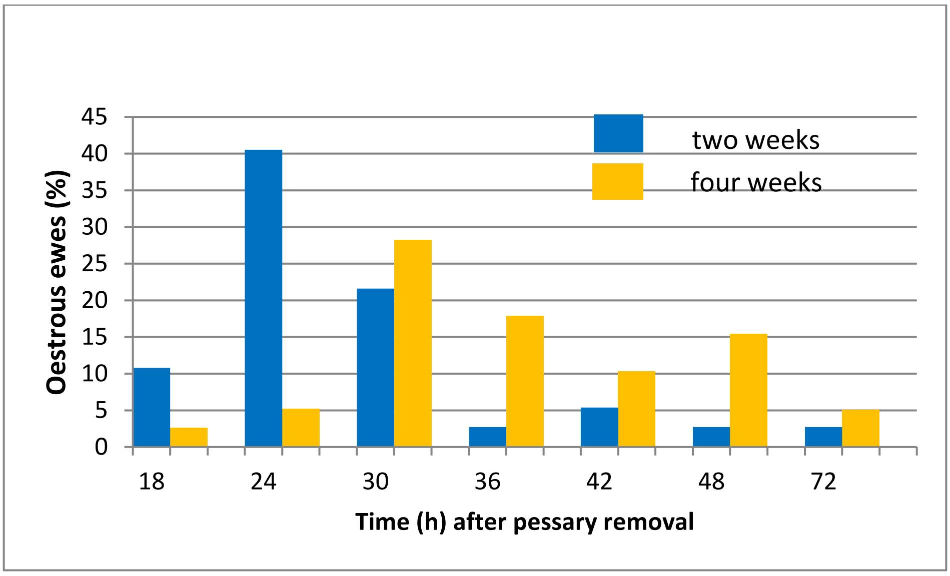
4. Double pessaries and duration of treatment

Given the low progesterone levels during pessary treatment, observations were made to determine if the number of pessaries (one versus two) or duration of treatment (two versus four weeks) influences the timing of oestrus and pregnancy rate. Treatment groups were one pessary for two weeks (Control), two pessaries for two weeks, one pessary for 28 days replaced after 14 days and two pessaries for 28 days both replaced after 14 days.

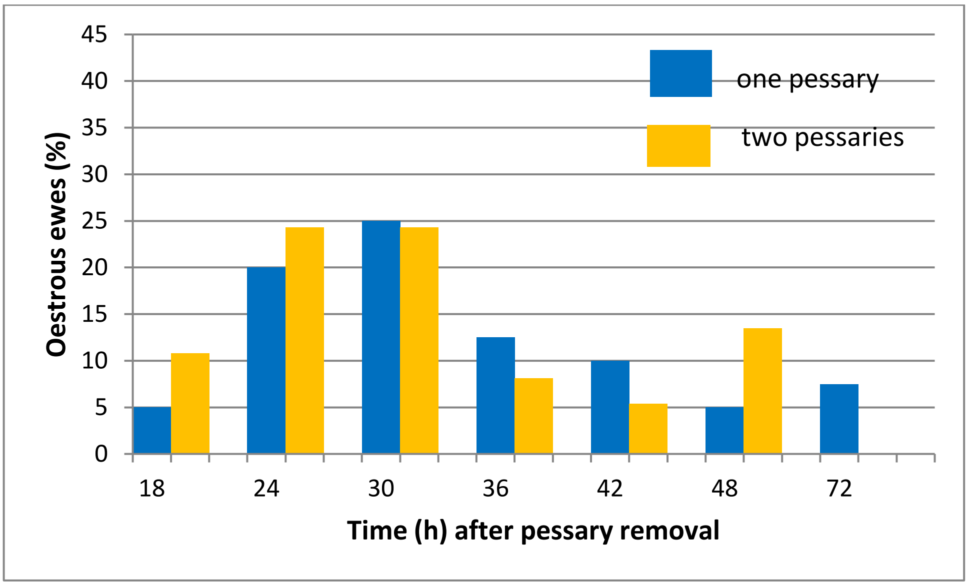
Treatment for four weeks significantly delayed the onset of oestrus compared with two weeks, irrespective of the number of pessaries (Figure 8a). Treatment with either one or two pessaries did not influence the timing of oestrus irrespective of the duration of the treatment (Figure 8b). Treatment for four weeks significantly reduced pregnancy rate compared with two weeks (39.0% and 56.5%) but pessary number had no significant effect (43.8% versus 53.8% for one and two pessaries respectively). There were no significant effects of either treatment on litter size

Figure 8. Effect of duration of treatment (two or four weeks) and number of pessaries (one or two) on time of onset of oestrus following pessary removal (in the four-week treatments, pessaries were replaced after two weeks)

(a) Two weeks versus four weeks



(b) One versus two pessaries



In conclusion, treatment for four weeks delayed the onset of oestrus and reduced pregnancy rate whilst the use of two pessaries (rather than one) had no significant effect.

Strategies to improve AI success rates – changes to flock management practises

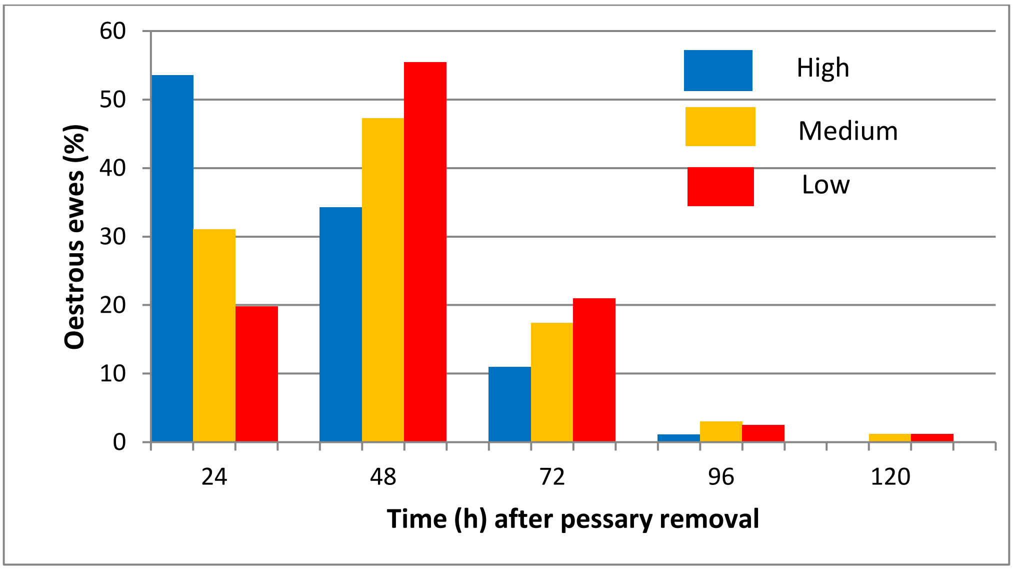
1. **Long and short term nutrition**

   It takes approximately six months for follicles to fully mature and this period corresponds approximately with the interval between the previous lambing/lactation and the cycle of AI. Nutrition during this period – **long term nutrition** – influences the success of AI programs. The benefits of high nutrition (BCS 4.0$^+$) when compared with either medium (BCS 3.3$^+$) or low (BCS 2.7$^+$) nutrition are:

   - Oestrus occurs earlier (Figure 9) – hence insemination should commence no later than 42h after pessary removal in relevant flocks.
   - More ewes come into oestrus (e.g. 91.9% versus 85.2% and 85.7%).
   - Pregnancy rates are higher (e.g. 81.1% versus 71.1% and 73.7% using chilled semen).
   - Litter size is higher (e.g. 1.50 versus 1.35 and 1.28).

   On the other hand, high nutrition (1.75M) during the pessary period – **short term nutrition** - increases pregnancy rate (e.g. 79.0% versus 72.3% for the 1.0M group) but there is no effect on either time of oestrus or litter size. The reasons for the higher pregnancy rate are not known but may be associated with improvements in oocyte/embryo quality.

Figure 9. Time of onset of oestrus following pessary removal in ewes fed either a high (BCS 4.0$^+$), medium (BCS 3.3$^+$) or low (BCS 2.7$^+$) diet between the previous lambing/lactation and the cycle of AI.

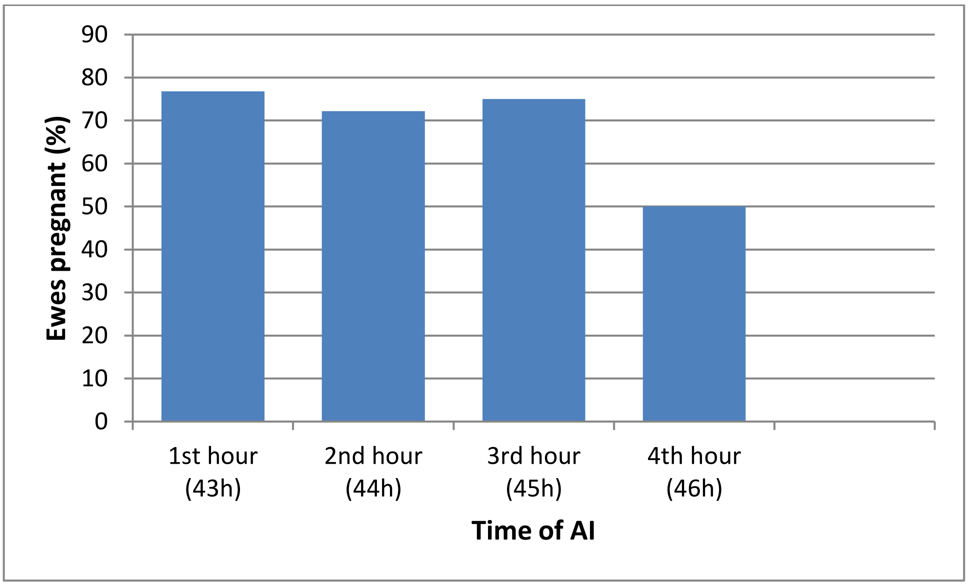


Whilst maintaining high levels of nutrition can be challenging, particularly if Spring pastures are poor, these results indicate the importance of nutrition during the months leading up to the AI program as well as during the weeks of synchronisation. Importantly, when determining the time of insemination, consideration should be given to the nutritional status of the flock.

2. **Managing ewes on the day of AI**

Both anecdotal and research evidence indicate that pregnancy rates can be higher in ewes that are inseminated earlier in the program. This is exemplified in data presented in Figure10 where pregnancy rates declined in ewes that were inseminated after the first three hours.

Figure 10. Effect of time of insemination on pregnancy rate (insemination with frozen-thawed semen commenced at the start of the first hour, approximately 42h after pessary removal).



The reasons for this decline are not clear - it is highly unlikely that semen quality is involved but it may be related to an increase in the number of ewes ovulating later in this period. Alternatively, time–critical exposure to pheromones produced by a large number of cycling sheep may be responsible. Until these data are validated and better understood, it is recommended that pessary removal in large programs be staggered in groups on a three-hourly basis.

3. Using teaser marks

Given the variability in patterns of oestrus, information provided by the use of teasers can be useful in helping to overcome this problem.

(a) Timing of AI

Generally, insemination commences at a set time – about 42h after pessary removal with CIDRs and about 48h with sponges. Until variability in the patterns of oestrus can be better controlled, one strategy is to commence insemination at a time indicated by the first occurrence of oestrus. With CIDRs, this is usually in the 18 -24h period after pessary removal - many of these ewes will ovulate from about 42 - 44h. A later onset – from 30h – necessitates a later insemination time. Having the flexibility to either advance or delay timing to better match the pattern of oestrus is likely to improve pregnancy rates although there are numerous logistical constraints to this strategy.

(b) Inseminate in order of oestrus occurrence

The ability to inseminate ewes in approximate order of oestrous onset is a worthwhile strategy. Whilst drafting ewes on the day of AI is generally undesirable, identification of ewes within early, late and intermediate categories will result in a better alignment of insemination and ovulation times.

(c) Not inseminating unmarked ewes

In most AI programs up to 20% of ewes are not marked by teasers at the time of insemination. Their insemination usually results in a pregnancy rate of 30 – 40%. A decision to inseminate these ewes should be based on the value of lambs expected given the costs involved.

Other observations of interest

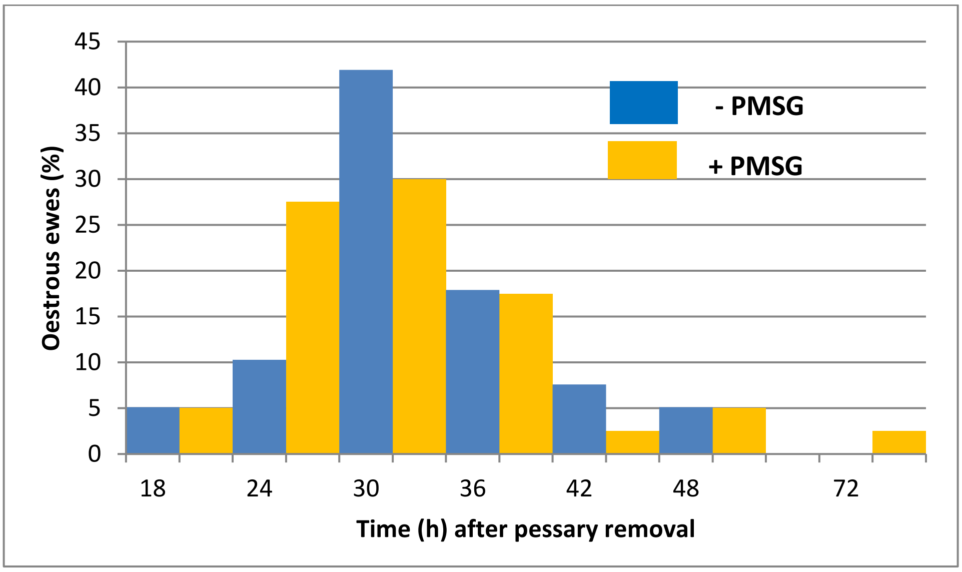
1. Dose of PMSG

Observations in Spring indicated that a higher dose (500 i.u. versus 400 i.u.) significantly increased litter size (1.50 versus 1.13). Slightly more ewes treated with 500 i.u. were in oestrus at 24h (11.7% versus 4.0%) and, overall, slightly fewer ewes were not marked by teasers (8.5% versus 13.9%) compared with ewes treated with 400i.u.

2. Synchrony of oestrus without PMSG

Data presented in Figure 11 indicate that a good synchrony can be obtained without PMSG treatment. Overall, 87.2% and 87.5% of the –PMSG and +PMSG groups were detected in oestrus. Despite this, the use of PMSG is recommended because of the likely benefit in synchronising oestrus in situations where follicle growth is compromised (e.g. poor nutrition, heat stress).

Figure 11. Timing of oestrus after pessary removal in ewes treated or not treated with PMSG (400 i.u.).



3. Occurrence of a “pseudo-oestrus”

Ewes can display normal oestrous behaviour without attracting the attention of teasers. Some of these ewes will be marked eventually by the teasers. It is speculated that this “pseudo-oestrus” results from inadequate or delayed follicle development resulting in deficiencies in the pheromonal cascade necessary for teaser stimulation.

4. Comparison of teasers treated with testosterone for either two or four weeks

Wethers treated with testosterone for two weeks often appear to quickly lose interest. Wethers treated for either two or four weeks were compared with entire rams in their ability to detect oestrous ewes. The patterns of oestrus were similar although the longer treatment appeared to improve teaser vigour.

What is the best treatment protocol?

There are a number of variations to the conventional protocol that are worth considering. Two of these have resulted in significant increases in fetuses/100 ewes inseminated – treatment with PMSG 12h before pessary removal (17% increase) and pre-treatment with PGF2α (35% increase). One constraint is that both rely on the presence of cycling ewes and the extent to which they are useful in Spring remains to be determined. This issue will be addressed in the Spring of 2021. In addition, on-farm evaluations of these protocols are planned for 2021/22 season. Summaries of the current options are outlined below.

- **Conventional protocol** (single pessary and PMSG)
    - Can produce good results but is unreliable due to large variation in the pattern of oestrus (particularly with ewes coming into oestrus late) and associated problems with follicle quality.
    - It is recommended that 500 i.u. PMSG be used.
    - The preferred timing of insemination should be indicated by the pattern of oestrus (largely based on observations at 24h) – an "on time" oestrus requires insemination from 42h whereas a delayed oestrus necessitates insemination from 48h.

- **Replacement of pessary on day 9**
    - This option uses the conventional protocol but with the pessary being replaced on day 9.
    - Treatment results in a delayed onset of oestrus with insemination needing to commence at 48h.
    - This delay may be useful in some flocks provided the timing of insemination is adjusted.
    - Whilst further research is required, this protocol might best suit flocks with a history of poor synchrony of oestrus or in flocks experiencing nutritional stress.

- **Treatment with PMSG 12h before pessary removal**
    - This option uses the conventional protocol but with PMSG given 12h before pessary removal.
    - It is likely that treatment improves the quality of ovulatory follicles that develop late in the pessary period.
    - Treatment results in an earlier onset of oestrus with insemination needing to commence 42h after pessary removal.
    - Increases in both pregnancy rate and litter size result in worthwhile increases in potential lambing rate (e.g. 107.7% versus 90.9%).
    - Likely to improve the outcome in flocks with a history of poor performance, particularly those that experienced poor/delayed onset of oestrus.
    - The efficacy of this treatment in Spring is not known.

- **Pre-treatment with CIDRs or PG**
    - This option is based on the conventional protocol but uses either CIDR or PG pre-treatment to better control ovulatory follicle growth during the AI cycle.
    - Both pre-treatments improve pregnancy rate but only the PG pre-treatment increases the potential lambing rate (e.g. 140.0% versus 106.4%).
    - Insemination at 42h after pessary removal is recommended.
    - Both the PG and CIDR options are likely to be beneficial in "problem" flocks.
    - This protocol is yet to be adapted for used in Spring.

- **Combination of PMSG treatment and PG pre-treatment**
    - This combination is yet to be examined although it is expected that pre-treatment precludes the need to administer PMSG early.
    - Insemination will need to commence 42h after pessary removal.

- **PG treatment immediately preceding a 7-day pessary period**
    - This protocol has not been examined in this project but is being used overseas.
    - Treatment improves the control of follicle growth compared with the conventional protocol.
    - The likely preferred time of insemination is 48h after pessary removal.

Endnote

Some additional studies remain to be conducted including the evaluation in Spring of the two more promising protocols and their on-property assessments. In addition, both progesterone and oestrogen concentrations are to be determined. This document will be updated as these results become available.

Appendix Table 1. Treatment protocol for a conventional AI program using CIDRs/PMSG.

Day	Activity	Comments
1	Inject wethers (n=10% of ewes) – 2ml Ropel	Ropel* requires a weekly injection (subcutaneous); other products are available
8	Inject wethers – 2ml Ropel Insert CIDRs	
15	Inject wethers – 2ml Ropel	
22	Inject wethers – 2ml Ropel	
23		
24		
25		
26		
27		
28		
29 **2 p.m**.	Inject wethers (2ml Ropel) and harness. Remove CIDRs, inject 500 i.u. PMSG. Run ewes and teasers together from CIDR removal.	Instead of harnesses, paint brisket with branding fluid. To avoid marks from initial activity, consider delaying harnessing/painting for several hours after exposure. Stagger CIDR removal by 3h in large programs.
30 2 p.m.	Observe mounting activity (24h after CIDR removal).	Teaser activity and number of ewe groups (harems) seeking attention indicate normality of synchrony. Consider delaying insemination (if possible) should oestrus be delayed.
31 **8 a.m.**	Commence AI (42h after pessary removal). Record oestrous marks at 2 p.m. (optional)	Option of drafting off marked ewes and inseminating first. Option of not inseminating unmarked ewes or using fresh semen if available.

**Ropel can be given over a two-week period; in this example, it is given over four weeks because the longer treatment induces better male-like activity.*

Appendix Table 2. Treatment protocol for an AI program in which ewes are pre-treated with CIDRs.

Day	Activity	Comments
1	Insert first CIDRs	
8	Inject wethers (n=10% of ewes) – 2ml Ropel	Ropel* requires a weekly injection (subcutaneous); other products are available
14	Remove first CIDRs Inject wethers – 2ml Ropel	
21	Insert second CIDRs Inject wethers– 2ml Ropel	
22		
23		
24		
25		
26		
27		
28	Inject wethers – 2ml Ropel	
29		
30		
31		
32		
33		
34 **2 p.m.**	Inject wethers (2ml Ropel) and harness. Remove CIDRs, inject 500 i.u. PMSG. Run ewes with teaser wethers from CIDR removal	Instead of harnesses, paint brisket with branding fluid. To avoid marks from initial activity, consider delaying harnessing/painting for several hours after exposure. Stagger CIDR removal by 3h in large programs.
35	Observe mounting activity (24h after CIDR removal).	Teaser activity and number of ewe groups (harems) seeking attention indicate normality of synchrony. Consider delaying insemination (if possible) should oestrus be delayed.
36 **8 a.m.**	Commence AI (usually 42h after pessary removal). Record oestrous marks at 2 p.m. (optional)	Option of drafting off marked ewes and inseminating first. Option of not inseminating unmarked ewes or using fresh semen if available.

**Ropel can be given over a two-week period; in this example, it is given over four weeks because the longer treatment induces better male-like activity.*

Appendix Table 3. Treatment protocol for an AI program in which ewes are pre-treated with PG.

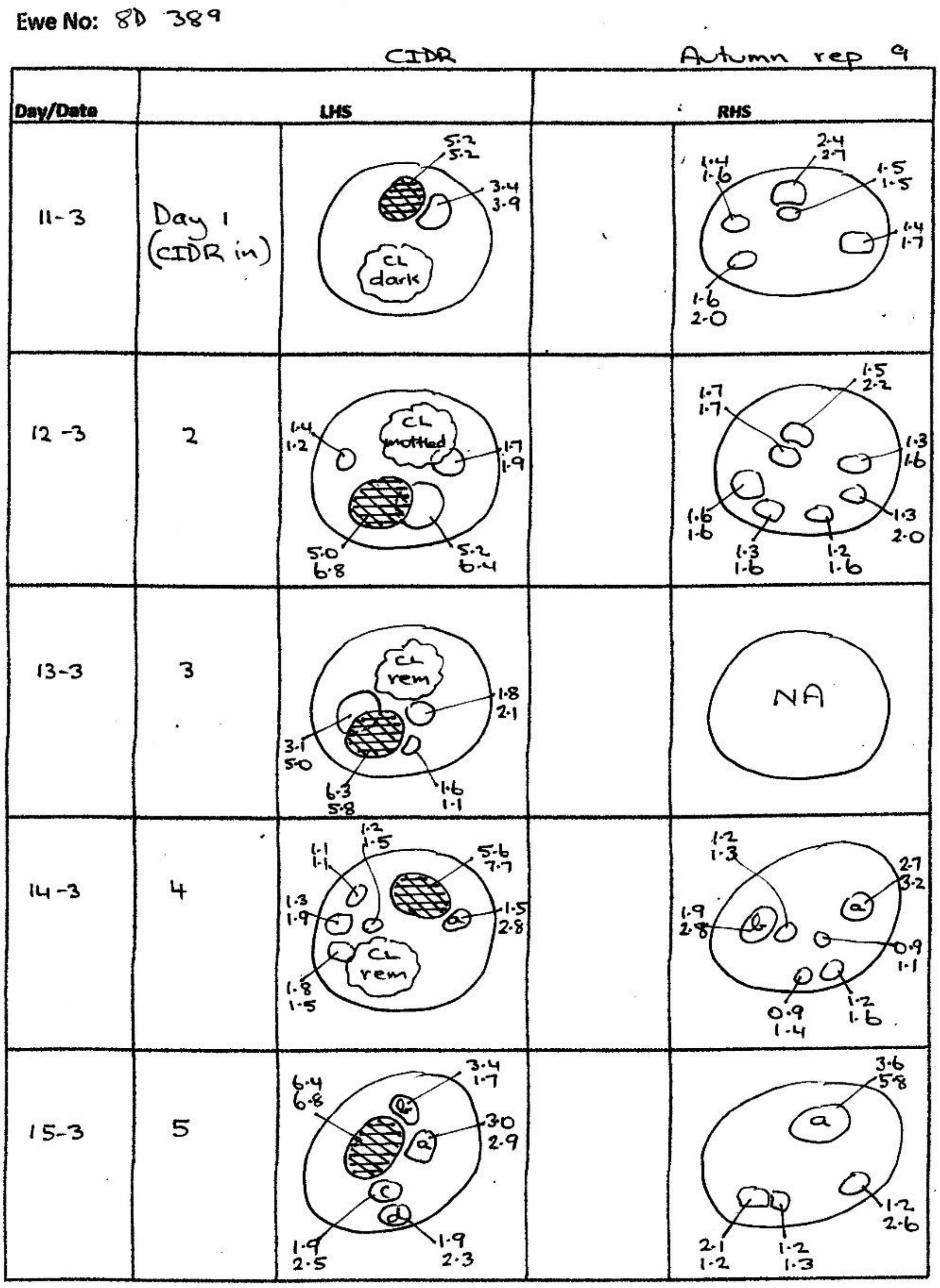
Day	Activity	Comments
1	Inject PGF2α (125 µg/ewe i.m.)	
2		
13	Inject wethers (n=10% of ewes) – 2ml Ropel	
20	Inject wethers (2ml Ropel)	Ropel* requires a weekly injection (subcutaneous); other products are available
21		
22		
25		
26		
27	Insert CIDRs Inject wethers (2ml Ropel)	
28		
29		
32		
33		
34	Inject wethers (2ml Ropel)	
35		
36		
37		
38		
39		
40 **2p.m.**	Inject wethers (2ml Ropel) and harness. Remove CIDRs, inject 500 i.u. PMSG. Run ewes with teasers from CIDR removal	Instead of harnesses, paint brisket with branding fluid. To avoid marks from initial activity, consider delaying harnessing/painting for several hours after exposure. Stagger CIDR removal by 3h in large programs.
41	Observe mounting activity (24h after CIDR removal).	Teaser activity and number of ewe groups (harems) seeking attention indicate normality of synchrony. Consider delaying insemination (if possible) should oestrus be delayed
42 **8a.m.**	Commence AI (usually 42h after pessary removal). Record oestrous marks at 2 p.m. (optional)	Option of drafting off marked ewes and inseminating first. Option of not inseminating unmarked ewes or using fresh semen if available.

**Ropel can be given over a two-week period; in this example, it is given over four weeks because the longer treatment induces better male-like activity.*

If PMSG is given early (-12h) in any of these protocols, timings need to change. One option is to remove pessaries at **6 p.m**. with PMSG being given at **6 a.m**. on that day. Insemination would then commence at noon (42h after pessary removal). Alternatively, if insemination needs to start earlier, pessaries can be removed at mid-day with PMSG being given the previous mid-night. This would enable insemination to start early (6 a.m. = 42h after pessary removal).

Attachment. Follicle maps

Follicle maps are constructed from ultrasound videos of the ovary and provide a means of monitoring follicle development and luteal regression. In this example, the ovulatory follicle (hatched) was present on the day of pessary insertion (with luteal regression evident) and remained until ovulation occurred on day 16.



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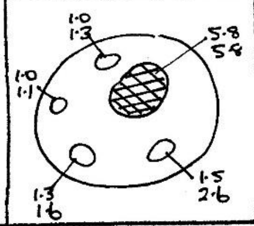
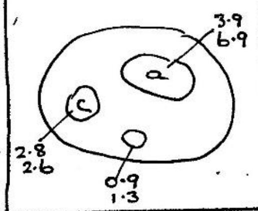
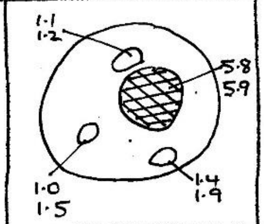
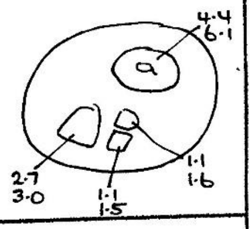
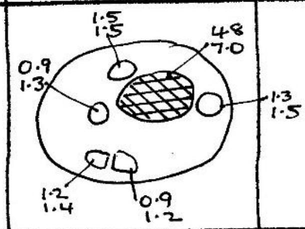
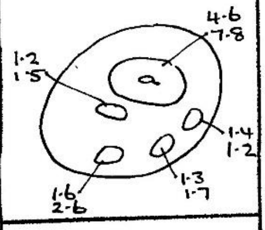
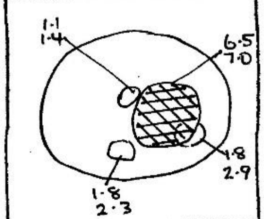
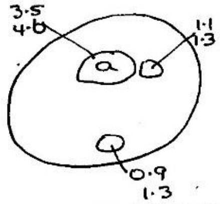
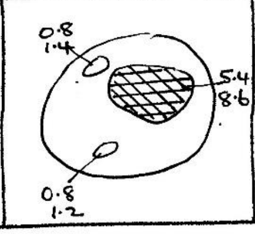
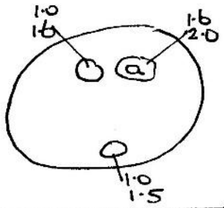
Day/Date	LHS			RHS
16-3	6			
17-3	7			
18-3	8			
19-3	9			
20-3	10			

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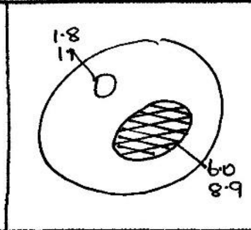
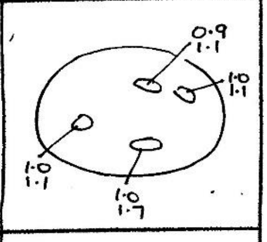
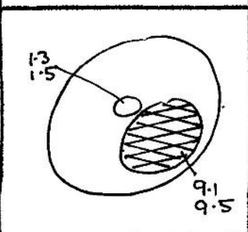
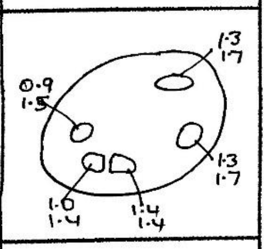
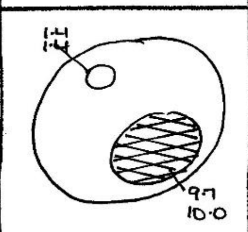
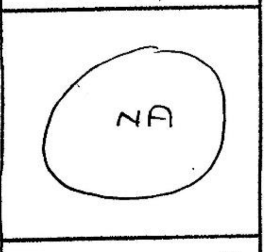
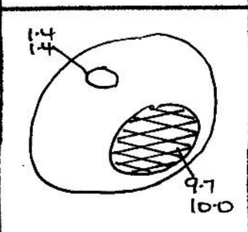
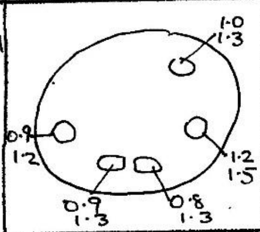
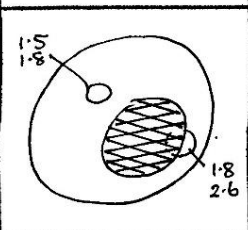
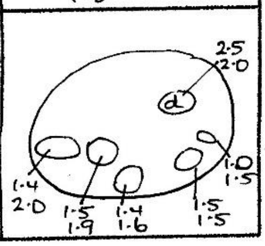
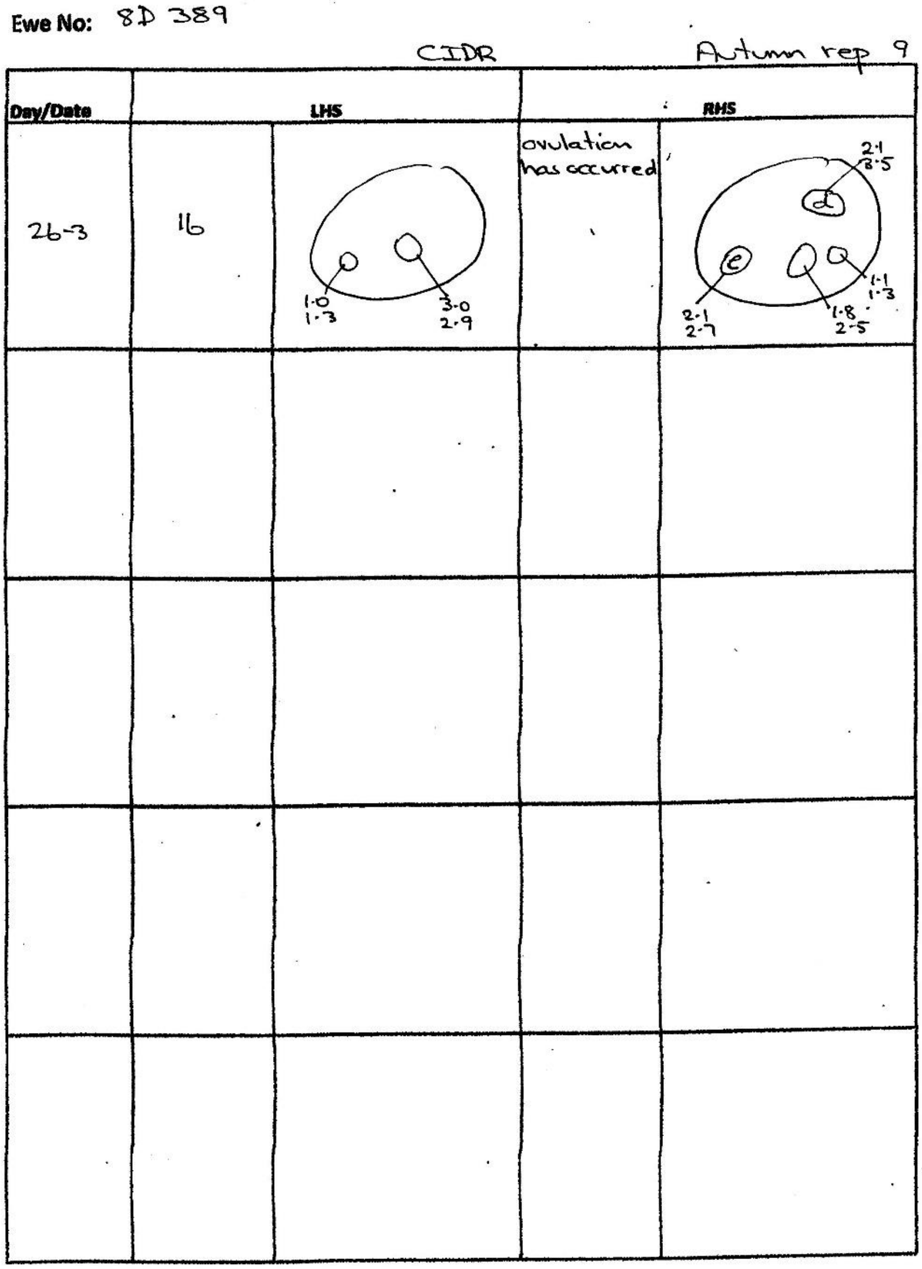
Day/Date		LHS		RHS
21-3	11			
22-3	12			
23-3	13			
24-3	14		day of CIDR removal	
25-3	15		in oestrus 9a.m. (21h)	



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Day/Date	LHS		RHS	
26-3	16	1·0 1·3; 3·0 2·9	ovulation has occurred	2·1 3·5; 1·1 1·3; 1·8 2·5; 2·1 2·7

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