Improving the success of sheep artificial insemination programs

A handbook for producers

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Summary

Poor synchrony of oestrus is the major cause of poor AI results in the Merino. In this document, background information on this problem is provided together with strategies to improve the success of AI programs. The research has identified that the progesterone pessary, whilst essential for synchrony of oestrus, adversely affect follicle growth and this affects both synchrony of oestrus and pregnancy rates. To overcome this problem, several modifications to the standard treatment protocol have been investigated with the most promising resulting in an increase in the number of fetuses per 100 ewes inseminated of 33.9%. On-farm evaluation of these new protocols will be conducted in the next two years. In addition, there are other "management" factors that affect the success of AI, the most notable being incorrect timing of insemination. Determining the correct timing of insemination is a challenge because the patterns of oestrus are very variable being affected by multiple factors including time of year and nutrition. It is expected that the modifications to the treatment protocol will eliminate much of this variability. Strategies the producer can adopt to maximise the likelihood of AI success are discussed together with information on how to troubleshoot a problem AI program.

Background

Evidence collected over several decades indicates that the success of AI in the Merino has remained highly variable with little or no improvement. This assessment is supported by findings of a SASBMA survey of the 2011 and 2012 seasons. Of the 32 respondents representing 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 primarily responsible.

The research funded by AWI Ltd aimed to improve synchrony of oestrus and to develop new strategies to improve the success rates of AI programs. Trans-rectal ultrasonography was used to examine what really happens to the ovary during the synchronisation procedure and these observations have resulted in the recommendations outlined in this document. In addition, steps producers can take to implement and monitor the well-being of the program are presented.

Patterns of oestrus

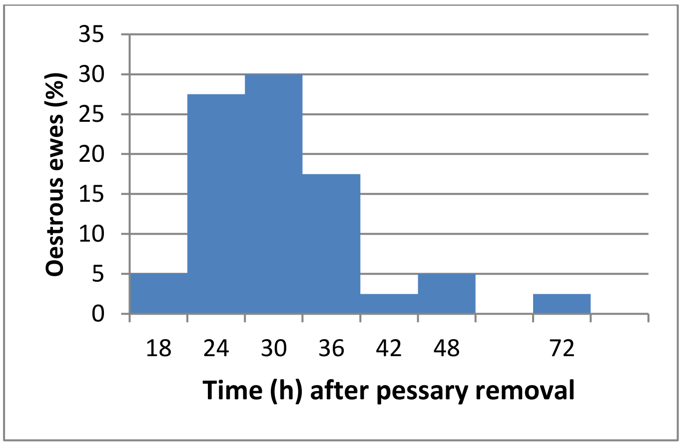
A major problem the AI industry has faced for many decades is the incorrect belief that pessary treatment results in a synchrony of oestrus that it adequate for fixed-time AI. This is not the case with poor synchrony being responsible for most failed AI programs. A good rule of thumb is that a good synchrony will result in a good pregnancy rate (provided the time of insemination is optimal).

Consequently, it is important for producers to become familiar with how variable the patterns of oestrus are (Figure 1).

Figure 1. Examples of variation in oestrous patterns after 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. Ewes were observed up to 48h with a final observation at 72h.

Flock 1 (total ewes in oestrus = 97.5%)

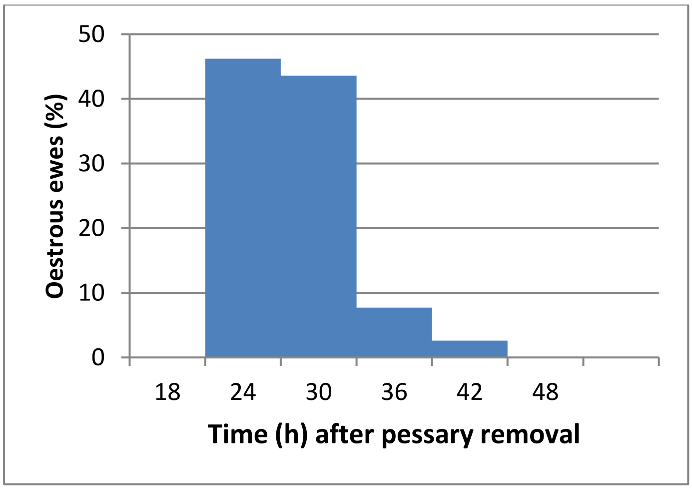
An example of a good synchrony that is normally associated with high pregnancy rates.



- 30.0% in oestrus at 24h
- 2.5% not detected in oestrus
- 2.5% in oestrus after 48h

Flock 2 (total ewes in oestrus = 97.5%)

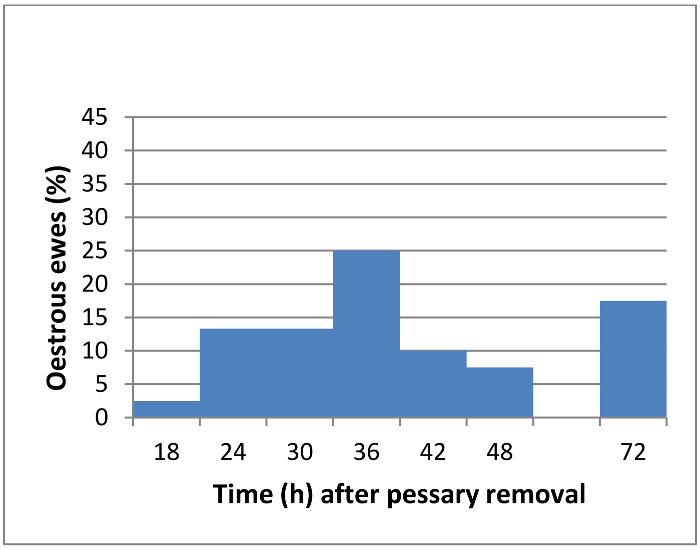
Another example of a good synchrony.



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

Flock 3 (total ewes in oestrus = 87.5%)

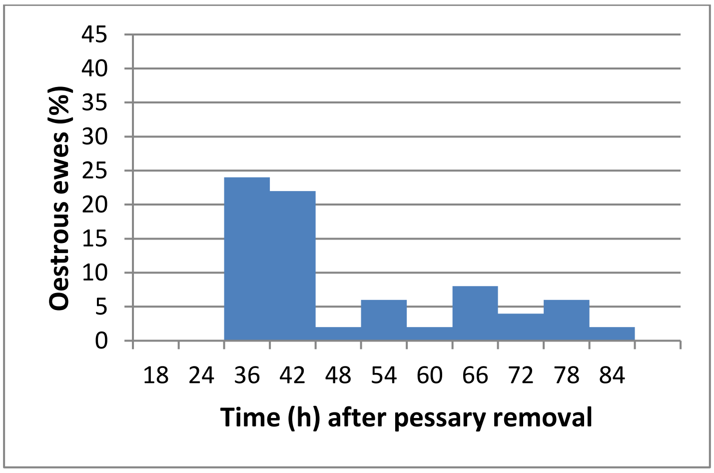
An example of synchrony spoilt by a significant number of ewes coming into oestrus after 48h (17.5%) or not coming into oestrus at all (12.5%).



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

Flock 4 (total ewes in oestrus = 74.0%)

An example of a very poor synchrony with no ewes in oestrus at 24h, less than half in oestrus at 48h and 26.0% not coming into oestrus. This pattern is associated with very low pregnancy rates.



- 0.0% in oestrus at 24h
- 26.0% not detected in oestrus
- 30.0% in oestrus after 48h

It is desirable that the pattern of oestrus be identified by the producer as soon as practicable after pessary removal. This can help the practitioner in knowing whether the timing of insemination is correct although last minute changes to the timetable are often not possible. This lack of flexibility is and probably will remain a constraint to the conduct of successful AI programs. The "normality" of oestrus can be assessed using the following criteria:

- Time when ewes are first observed in 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%. Absence of mounting activity at this time is indicative of a delayed oestrus.
- The percentage of the flock not detected in oestrus at the commencement of insemination. Generally, ewes that come into oestrus after this time have a reduced chance of getting pregnant.
- The percentage of the flock that ultimately fails to come into oestrus (up to 20%). Some of these ewes will conceive to AI (e.g. 30 – 40%) but a decision needs to be made on the economic worth of insemination.

It is important for producers to get familiar with the oestrous patterns of their flocks. Individual flocks can show significant variability between years and accumulated knowledge is valuable when planning and implementing future programs.

What really happens during pessary treatment?

It has been thought for 50 – 60 years that the pessary prevents follicle development and that after the pessary is removed, an ovulatory follicle develops in association with the onset of oestrus. In fact, whilst the pessary does prevent oestrus, it plays little or no role in controlling development of the ovulatory follicle.

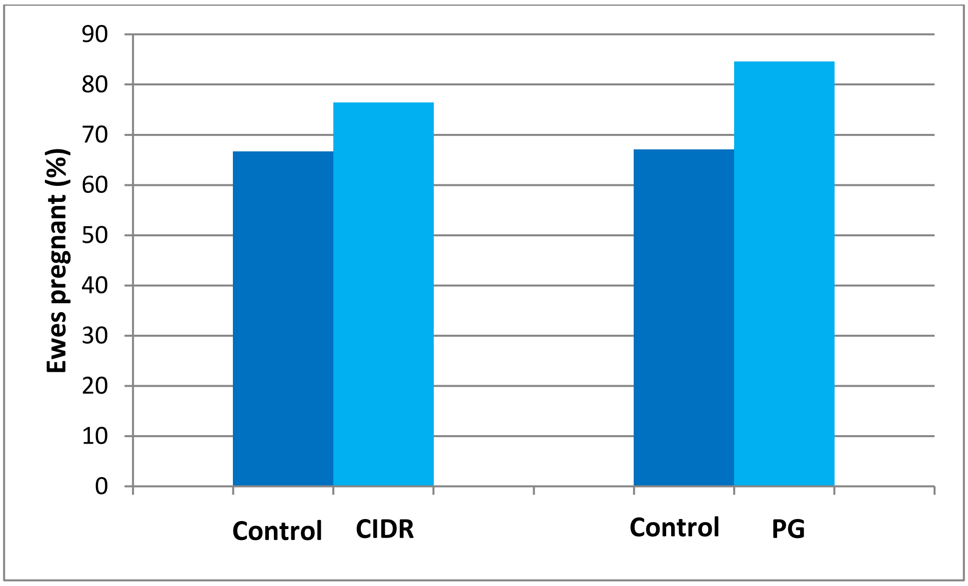
Using trans-rectal ultrasonography, it has been shown that the ovulatory follicle can emerge at any time during pessary treatment. As a result, when pessaries are removed, there is a wide range of ovulatory follicle ages (1 – 14 days) and this adversely impacts the success of AI programs. Ewes with young follicles (those that form late in the pessary period) come into oestrus later and with greater variability than ewes with older follicles. This is the situation in Autumn – the opposite occurs in late Spring/early summer. Ewes with ovulatory follicles that emerge mid-way through pessary treatment are the most fertile and show minimal variability in time of onset of oestrus. Additionally, one side effect of pessary treatment is a disproportionate number of ewes with young follicles and this further contributes to poor pregnancy rates (depending on time of year). The key to improving synchrony of oestrus and the success of AI programs in general, lies in the development of treatment protocols that are better able to control the age of the ovulatory follicle and hence follicle quality.

Strategies to improve success rates – changes to the treatment protocol

Substantial progress has been made in the development of improved protocols for synchrony of oestrus. Appropriate recommendations will be made as soon as the relevant on-farm evaluations are completed.

1. **Pre-treatment before pessary insertion**
   Pre-treatment with either a CIDR or prostaglandin (PG) leads to better control of follicle development during pessary treatment. Pre-treatment with a CIDR involves inserting an initial CIDR 21 days before the second CIDR whilst pre-treatment with PG involves one injection 27 days before the pessary is inserted. With both pre-treatments, pregnancy rates are improved (Figure 2).

Figure 2. The effect of pre-treatment with either a CIDR or prostaglandin (PG) on pregnancy rates following AI with frozen-thawed semen.

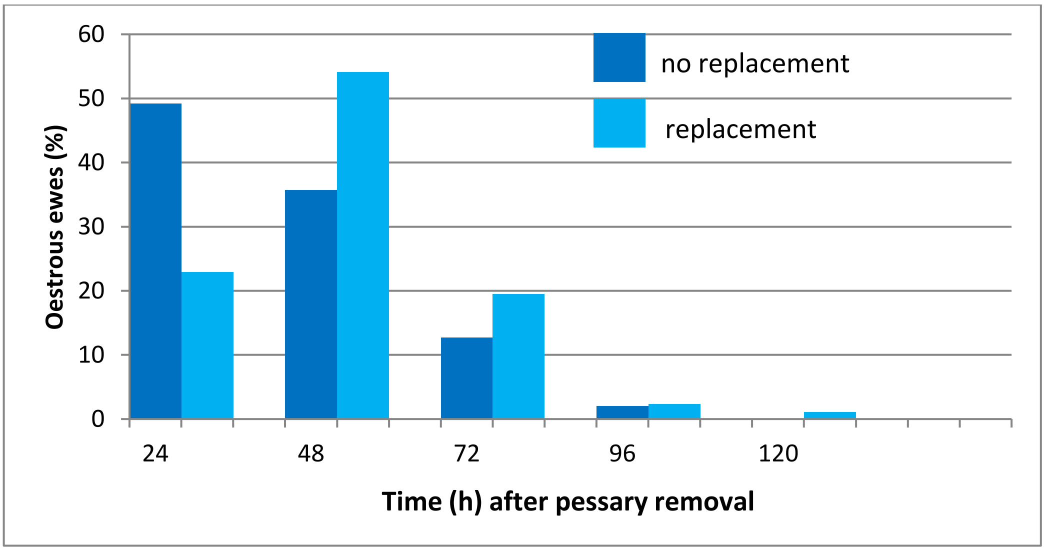


The potential lambing rate (fetuses/100 ewes inseminated) was not improved with the CIDR pre-treatment due to a reduction in litter size (a reduction most likely due to chance). However, this figure was increased by 33.9% with the PG pre-treatment and this was due to increases in both fertility and twinning rate.

2. **Replacement of pessaries on Day 9**

Although pessary replacement is an additional expense, it does improve the synchrony of oestrus (Figure 3) and this is potentially beneficial. However, the overall pattern is delayed and this necessitates a later time of insemination. Although further research is required, pessary replacement might be useful in flocks with a history of poor AI performance.

Figure 3. Effect of pessary replacement (Day 9) on timing of oestrus.



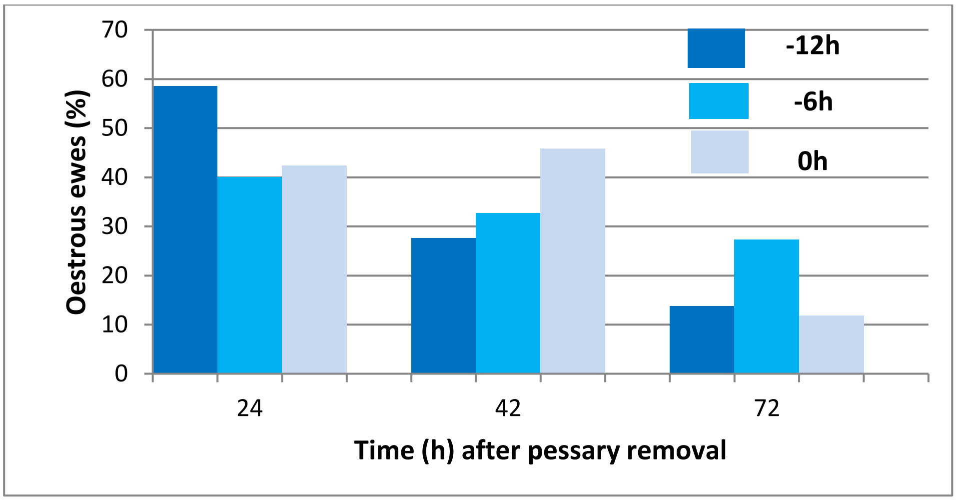
3. **Early treatment with PMSG**

The inability of young ovulatory follicles to produce sufficient oestrogen is a likely cause of poor synchrony following pessary removal (at least in Autumn). PMSG, which stimulates follicle growth, is usually given at pessary removal but treatment 12h beforehand results in an improved synchrony (Figure 4a) in association with a 17% increase in the number of fetuses per 100 ewes inseminated (Figure 4b). Because this treatment induces an earlier onset of oestrus, it is important that insemination commences about 42 – 43h after pessary removal.

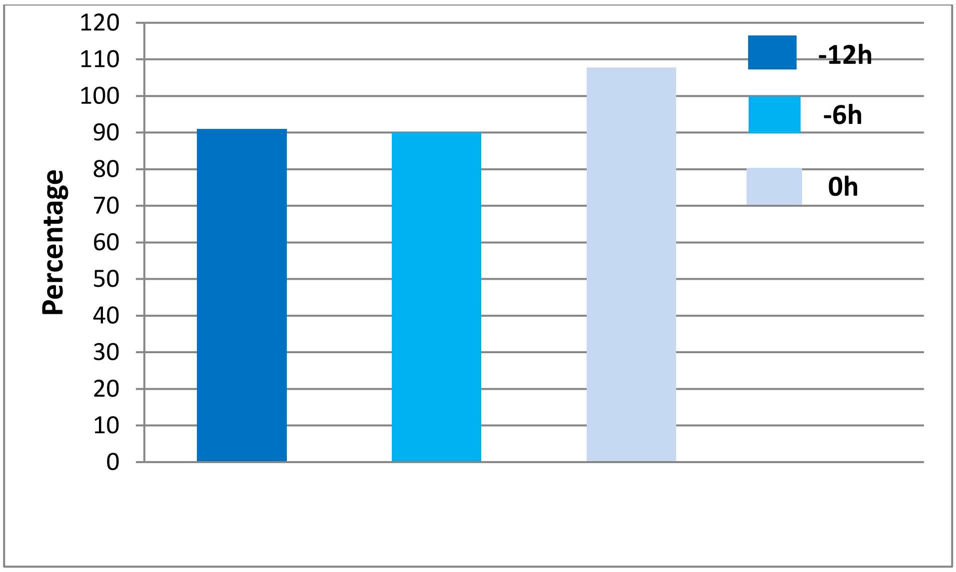
Some ewes treated early with PMSG display overt sexual behaviour (mounting teasers and other ewes) 24h after pessary removal indicating a more intense oestrus. Again, this approach has the potential to be useful in flocks that have a poor AI history, especially if a delayed oestrus is involved.

Figure 4. Effect of early PMSG treatment (-12h, -6h and 0h relative to pessary removal) on synchrony of oestrus and AI outcomes.

(a) Timing of oestrus.



(b) Fetuses/ewes inseminated



4. **Treatment with 500 i.u. PMSG rather than 400 i.u.**
   An increasing number of AI programs now use the higher dose and, whilst it does increase the cost, there is an increase in litter size and a small improvement in synchrony. This is another strategy to use in "problem" flocks.

Protocol details for (1) a standard program and (2) a PG pre-treatment program are provided in Appendix Tables 1 and 2 respectively.

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

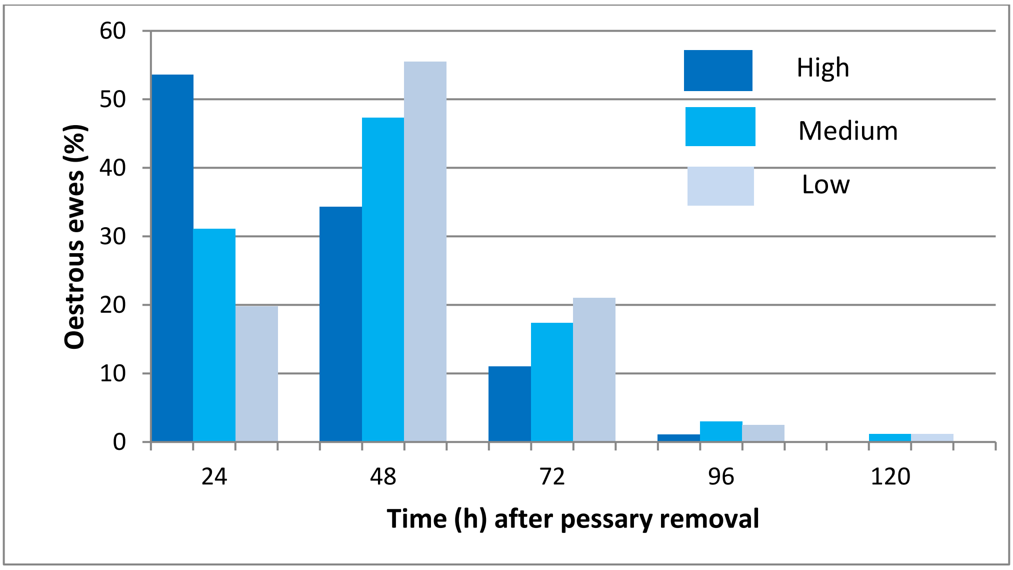
1. **Long and short term nutrition**

It takes approximately six months for ovulatory follicles to fully mature and this period corresponds 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 5) – hence insemination should commence no later than 42h after pessary removal in well-nourished flocks.
- More ewes come into oestrus (91.9% versus 85.2% and 85.7%).
- Pregnancy rates are higher (81.1% versus 71.1% and 73.7% using chilled semen).
- Litter size is higher (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 (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 it may be associated with improved follicle quality.

Figure 5. Timing 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.

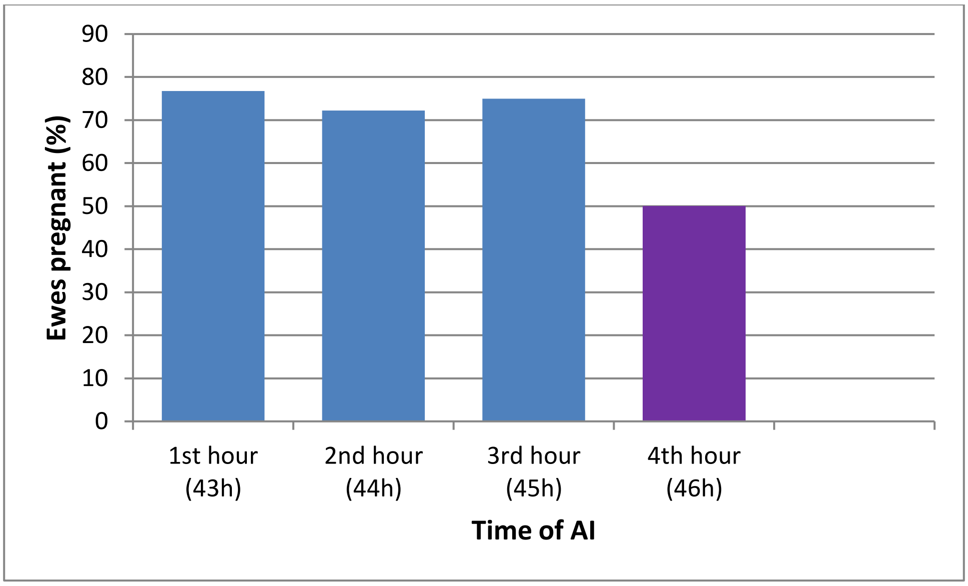


Whilst maintaining high levels of nutrition can be challenging, particularly if Spring pastures are poor, these results indicate the importance of nutrition leading up to and during the conduct of the AI program. An important point is high nitrogen diets (e.g. lucerne, clover, lupin grain) should be avoided from two weeks before to two weeks after AI. These diets can produce excess ammonia after consumption that adversely affect oocyte/embryo quality.

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 Figure 6 where pregnancy rates declined in ewes that were inseminated after the first three hours.

Figure 6. 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. One explanation is that ova, after ovulation, quickly lose their ability to fertilise. Until these data are better understood, it is recommended that pessary removal in large programs be staggered – to at least produce a.m. and p.m. groups.

3. **Using teaser marks**

Again, preparing teasers is an additional cost but the information they provide is invaluable.

- They indicate the normality of the synchrony, especially at 24h and this can lead to late adjustments in insemination time.
- Insemination of ewes in groups based on occurrence of oestrus (e.g. at 24h and 36h) is good practice. This avoids situations where ewes that ovulate early aren't inseminated until late in the day.
- Unmarked ewes are generally less fertile than marked ewes and a decision on whether they are worth inseminating needs to be made. Alternatively, these ewes can be inseminated with fresh semen should it be available.

In addition, teasers can play an important role in stimulating follicle development, particularly in Spring when a significant proportion of the flock are normally in anoestrus.

4. Maximising exposure to the ram effect

To maximise the ram effect, ewes can be shedded with but physically separate from entire rams for up to 6h after pessary removal. This exposure potentially improves follicle maturation including the ability of the follicle to produce oestrogen.

5. Culling dry ewes

Culling ewes known to have failed to lamb in the previously AI program should improve pregnancy rates.

Implementing an AI programs

In addition to the issues outlined above, other aspects that are important in implementing an AI program include:

- **Length of pessary treatment** – a 14-day period is standard and, given what is now know about follicle growth, there is little reason for change. Treatment periods of 12 or 13 days are also effective.
- **Timing of insemination** – as mentioned earlier, this is the most challenging aspect of implementing an AI program. Ideally, insemination needs to occur a few hours before ovulation. A rough rule-of-thumb is that ovulation occurs approximately 24h after the onset of oestrus. With CIDRs, insemination should commence 42 – 44h after pessary removal (this is earlier than the 48 – 50h timing used with

sponges). There needs to be some flexibility in the timing as indicated by the following:

- there is natural variability in the timing of oestrus with some flocks being "early" (centred around the 24 – 30h period) and others being "late" (centred around the 30 – 36h period).
- well-nourished flocks come into oestrus earlier than less-nourished flocks.
- maiden ewes come in to oestrus earlier than mature ewes.
- if pessaries are replaced (e.g. Day 9), onset of oestrus is delayed.
- if PMSG is given at -12h, onset of oestrus occurs earlier compared with conventional treatment.

Mounting activity at 24h after pessary removal is a guide to when insemination should occur – activity at this time indicates that insemination should occur at 42 – 44h whereas a lack of activity indicates the need for a later insemination (e.g. 48h). Late changes in timing are not always possible (depending on the practitioner's work schedule etc) and, with large programs in particular, there is very little flexibility.

- **Volume of semen** – there is a tendency for some producers to reduce the volume of semen per ewe so that more ewes can be inseminated. This strategy is only worthwhile with a good synchrony. It is also desirable that each uterine horn be inseminated when frozen-thawed semen is used.
- **Preparing teasers** – teasers (10% of ewe numbers) should be introduced to the ewes at pessary removal. It takes two weeks of testosterone treatment to induce male-like behaviour. A better response is generally obtained with a 4 – week treatment. Teasers should be fitted with harnesses and crayons or have briskets painted with branding fluid. To avoid inaccurate marks, run teasers with ewes for several hours before harnessing/painting of briskets.

- **Stress** – it is important that ewes are exposed to minimum stress, particularly between the times of pessary removal and AI. The use of dogs should be minimal and limited drafting is desirable.
- **Weather** – whilst chronic hot weather can adversely affect AI outcomes, very few programs are aborted because of it. Consistently hot weather from about three days before AI until three days afterwards can affect patterns of oestrus and pregnancy rates. However, heat stress needs to be unrelenting with minimal or no relief at night and this is uncommon in most sheep grazing areas of Australia. Adequate shade/water is essential. Similarly, extreme cold/wet weather in the days leading up to AI and shortly thereafter can adversely affect success rates particularly in recently shorn sheep. Shedding of shorn sheep, especially at night, is essential in inclement weather.

Challenges of conducting AI in Spring/early Summer

It is estimated that approximately 50% of flocks in Australia are inseminated in late Spring/early Summer. AI programs at these times face additional challenges because there are two groups of ewes – those that are cycling naturally and those that are in anoestrus and are induced to cycle. These two groups respond differently to pessary treatment but are treated as one flock. Unique features of these flocks include:

- The most fertile ewes are those that are in seasonal anoestrus at pessary insertion.
- The non-cycling ewes come into oestrus earlier than cycling ewes making an overall preferred time of insemination difficult. There is no easy way of detecting whether or not ewes are cycling at pessary insertion.
- Insertion of the pessary causes some ewes (cycling and non-cycling) to undergo spontaneous luteinisation of one or more large follicles (effectively an ovulation without the release of the egg).
- These luteinised follicles produce progesterone which probably improves synchrony.
- Unlike Autumn, the younger ovulatory follicles in cycling ewes are associated with an improved synchrony and the older follicles are associated with a poorer synchrony.
- Fortuitously, one side effect of pessary treatment is that it increases the proportion of cycling ewes with young ovulatory follicles and this presumably improves synchrony.
- Nutrition is usually good resulting in ewes generally coming into oestrus earlier than in late Summer or Autumn.

Developing improved protocols for late Spring/early Summer is difficult for the reasons outlined above. The new protocol using PG requires ewes to be cycling and so will be less effective in Spring than at other times. Similarly, the early PMSG treatment aims to improve the performance of ewes with young ovulatory follicles. This treatment is effective in Autumn but is likely to be less effective in late Spring/early Summer. Additional research is planned to address these issues.

Trouble shooting

Much of the troubleshooting focuses on the pattern of oestrus which should play an important role in AI programs. Factors to consider include:

- **No ewes in oestrus by 24h** – this is indicative of a delayed oestrus. This delay can be up to 6 – 12h and, ideally, time of insemination should be adjusted. The factor most likely to influence the pattern of oestrus is nutrition with poorer nutrition delaying the onset of oestrus (and requiring a later insemination).
- **Many ewes in oestrus at 24h** – this is generally not a problem provided insemination commences 42h after pessary removal. Ewes can be in oestrus as early as 18h and up to 50% can be in oestrus by 24h.
- **Less than 50% of ewes in oestrus at insemination** – this indicates a very poor synchrony and poor pregnancy rates can be expected. One option is to not inseminate unmarked ewes or to use fresh semen if available. Poor nutrition can cause a delay in the onset of oestrus and it is suspected that the grazing of oestrogenic pastures can have a similar effect.
- **Teasers not showing interest at 24h** – provided teasers have been properly treated with testosterone, this is indicative of a delayed onset of oestrus.
- **Teaser lethargy** – this is possible if teasers are treated for only two weeks. Longer treatment (e.g. four weeks) overcomes this problem.
- **Ewes in oestrus several days after AI** – these ewes are either very late coming into oestrus or have re-ovulated after the initial ovulation. These ewes won't get pregnancy to AI but may conceive to backup rams.
- **Ewes behaving as if in oestrus but not attracting the attention of teasers** – a small percentage of ewes (e.g. 5 - 7%) can behave in this

way. This is normal in a flock treated with pessaries – at least some of these ewes are marked eventually.

- **Ewes producing mucus but not marked by teasers** – should mucus be seen (e.g. when the ewe is in a recumbent position), its colour is indicative of the oestrous status of the ewe. Ewes with globular opaque mucus can be 12h or more before oestrus whilst ewes with clear viscous mucus are generally in oestrus or approaching oestrus.
- **Flocks with a high incidence of uterine fluid** – this condition can only be observed by the practitioner whilst inseminating. The incidence is seasonal, being more common in Spring and its presence can be (but not always) associated with the consumption of oestrogenic pastures. These ewes will not get pregnant to AI but may conceive to backup rams as the condition is temporary.

What is the best treatment protocol?

There are variations of the conventional protocol that can be considered. One of the current constraints is that they are based on observations of naturally cycling flocks (the "Autumn" protocols) and the extent to which they are useful in Spring remains to be determined. In addition, on-farm evaluations, planned for 2022/23, need to be completed before recommendations can be finalised. 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).
    - 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 (500 i.u. PMSG) 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 performance or are poorly nourished.

- **Treatment with PMSG 12h before pessary removal**
  - This option uses the conventional protocol but with PMSG being given 12h before pessary removal.
  - 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 synchrony of oestrus.

- **Pre-treatment with PG**
  - This option is based on the conventional protocol but uses PG as a pre-treatment to better control follicle growth during the AI cycle.
  - This protocol has produced a 33.9% increase in the number of fetuses per 100 ewes inseminated.
  - Insemination at 42h after pessary removal is recommended.
  - This option is likely to be beneficial in "problem" flocks with poor/delayed synchrony of oestrus.

- **Combination of one or more treatments**
  - The most likely preferred combination is the use of early PMSG treatment and PG pre-treatment.
  - Insemination will need to commence 42h after pessary removal.
  - This combined option has not yet been examined.

- **PG treatment immediately preceding a 7-day pessary period**
  - This protocol has not been examined in this project but is increasingly 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.

Appendix tables 1 and 2 outline details of the conventional protocol and the modified protocol using PG pre-treatment respectively.

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 wethers 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. Daily tasks of an AI program in which ewes are pre-treated with PG.

Day	Activity	Comments
1	Inject PG (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; 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 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.
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 give PMSG at 6 a.m. and remove pessaries at 6 p.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 on day 24 (6 a.m. = 42h after pessary removal).