# Improving the success of sheep artificial insemination programs

## A handbook for practitioners

Prepared by: Simon Walker, Jen Kelly, Alice Weaver and David Kleemann

South Australian Research and Development Institute, Turretfield Research Centre, Rosedale SA 5350

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For further information:

walkersa@adam.com.au

dave.kleemann@sa.gov.au

alice.weaver@sa.gov.au

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**Summary**

Research outlined in this document aimed (1) to improve the success of fixed-time AI programs in sheep and (2) to determine why pregnancy rates have often been disappointing. The underlying assumption of the research was that the use of the progesterone pessary, whilst essential for synchrony of oestrus, adversely affects follicle development resulting in poor synchrony and reduced pregnancy rates. It was found that pessary treatment failed to prevent the untimely development of ovulatory follicles and, as a consequence, these follicles varied widely in age at pessary removal. This variability influenced both the timing of oestrus and pregnancy rates. Older ovulatory follicles were associated with an improved synchrony in Autumn but, in late Spring, an improved synchrony was obtained with young ovulatory follicles. Pregnancy rates were highest when ovulatory follicles emerged during Days 7 – 9 of the pessary period. New protocols based on these findings have undergone preliminary evaluation. The most promising, involving pre-treatment with PGF2α to control follicle emergence, increased the number of fetuses per100 ewes inseminated by 33.9%. Other factors identified as being responsible for poor AI results include highly variable patterns of oestrus and incorrect timing of insemination. Of these, improving synchrony of oestrus is the most challenging given that it is influenced by several factors, the most important being nutrition and time of year. This study has exposed numerous flaws in the implementation and conduct of AI programs and it is expected that the adoption of appropriate recommendations will result in improved AI pregnancy rates.

## BACKGROUND INFORMATION AND RESEARCH FINDINGS

### 1. Introduction

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 – 2022 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 indicated that the success of AI programs has remained highly variable with little or no improvement. 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 disappointing results. These data indicated 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 pessary treatment. From the data collected, new information on the relationships between follicle growth patterns, emergence of ovulatory follicles and reproductive outcomes following AI has been obtained. This information has led to (1) an understanding of the adverse effects that progesterone treatment has on ovarian function, (2) an awareness of the importance that age of the ovulatory follicle has on pregnancy rates, (3) a better understanding of the factors that affect synchrony of oestrus and (4) knowledge on how season influences AI outcomes. More importantly, the research had led to the development of new treatment protocols for synchrony of oestrus.

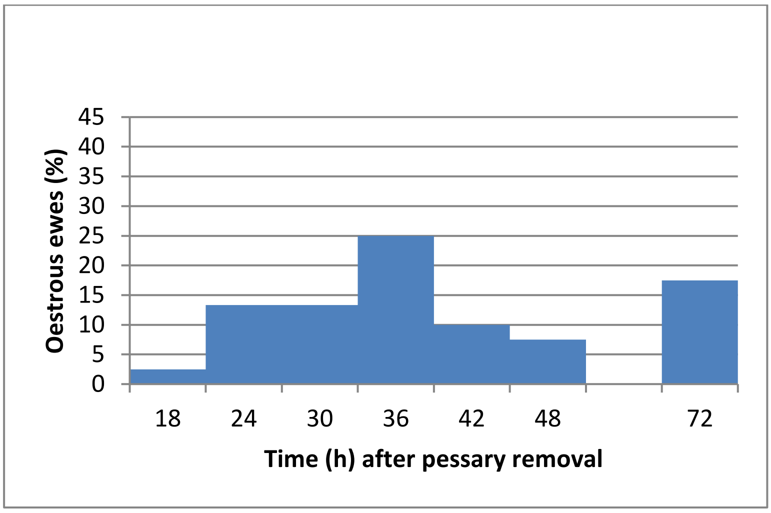
## 2. Variations in patterns of oestrus

Understanding the challenges of AI requires an awareness of the variability in the patterns of oestrus following pessary treatment. With the conventional 14-day pessary treatment, patterns of oestrus range from being very good to being very poor (Figure 1a - e) raising the question of what factors are responsible and what is a "normal" oestrous? Normality can be assessed using the following criteria:

- Time when the first oestrous ewes are detected. Observation 24h after pessary removal is very worthwhile. 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 is indicative of a delayed synchrony and this has ramifications for the timing of insemination.
- The percentage of the flock not detected in oestrus at the commencement of insemination (this should be 42 - 44h after pessary removal in most flocks). 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%). Overall, 30 – 40% of ewes that fail to be detected in oestrus conceive to AI.

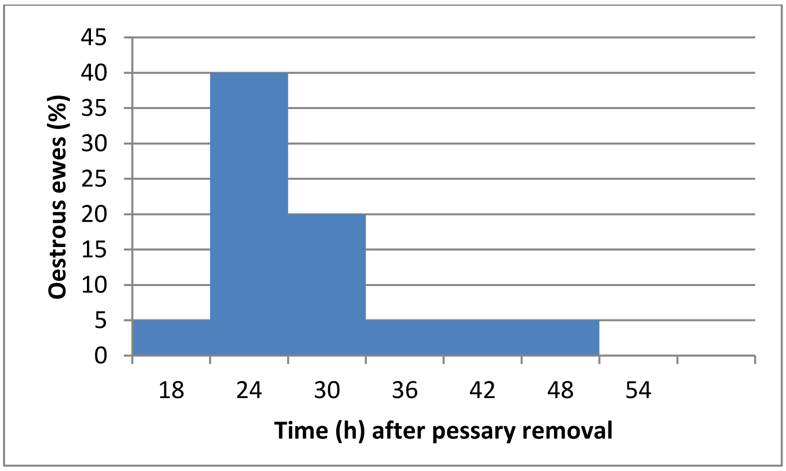
**Figure 1. Examples of timing of onset of oestrus following pessary removal**. These charts plot the percentage of new ewes in oestrus (using teasers with harnesses and crayons) at 6-h intervals after pessary removal. Observations were conducted up to 48h after pessary removal with a final observation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

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



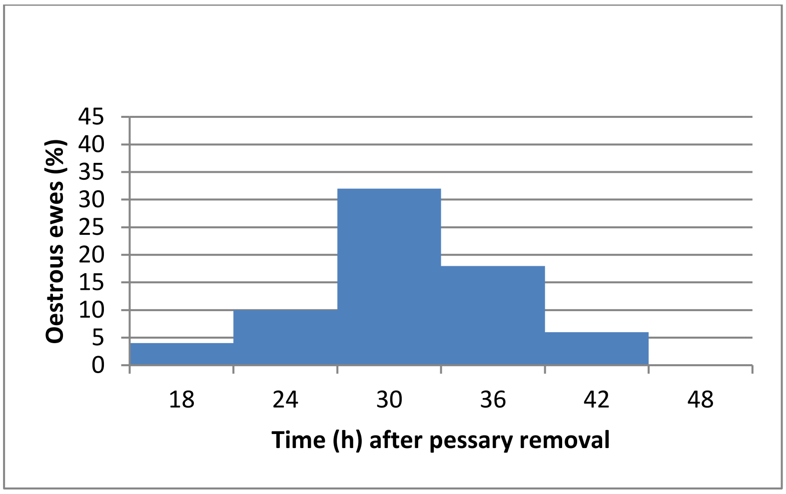

- 15.0% in oestrus at 24h
- 12.5% not in oestrus
- 17.5% in oestrus after 48h

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



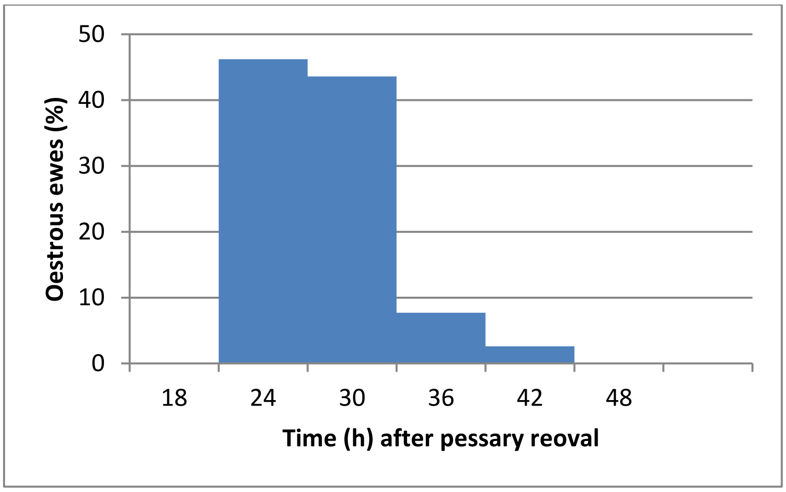

- 45.0% in oestrus at 24h
- 20.0% not in oestrus
- 0.0% in oestrus after 48h

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



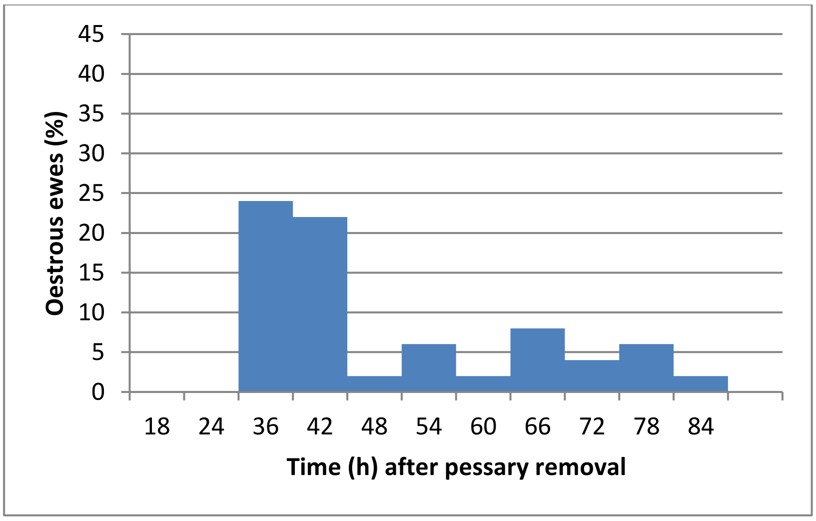

- 14.0% in oestrus at 24h
- 30.0% not in oestrus
- 0.0% in oestrus after 42h

d. **Flock 4 (sponge; ewes in oestrus = 97.5%)**




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

e. **Flock 5 (sponge; ewes in oestrus = 76.0%)**




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

Of these patterns, Flocks 4 is an example of a very good synchrony and a very good pregnancy rate can be expected provided the time of insemination is optimal. On the other hand, Flock 5 is an example of a very poor synchrony and a very poor pregnancy rate is almost guaranteed.

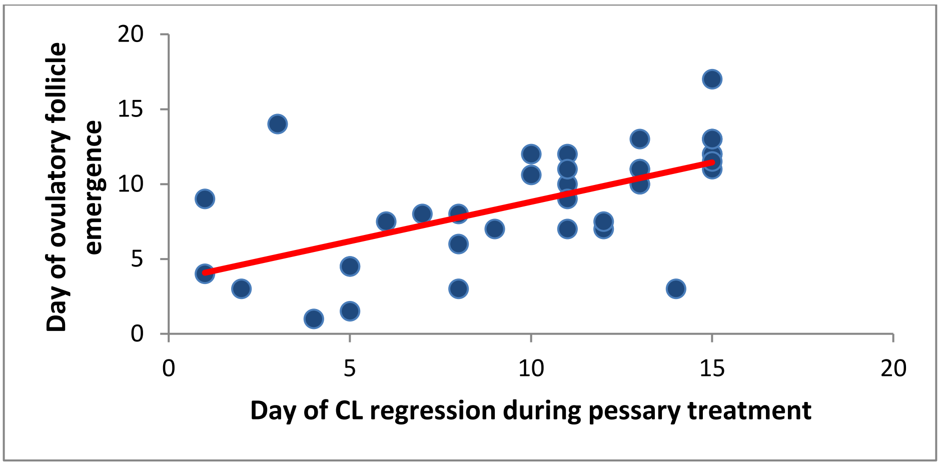
**Synopsis**. Given that the pattern of ovulation mirrors the pattern of oestrus (with about a 24h delay), variability of this magnitude highlights the difficulty in using a standard time of insemination across flocks. Ideally, time of insemination is adapted to suit the pattern of oestrus but this is logistically difficult if not impossible. The alternative approach is to develop a treatment protocol that guarantees a good synchrony and this is one focus of this study. A useful rule-of-thumb is that a very good synchrony will result in a very good pregnancy rate irrespective of semen quality (provided timing of insemination is correct).

### 3. The effect of pessary treatment on the quality of the ovulatory follicle

A question that has never been answered until now is whether pessary treatment, whilst essential for synchrony of oestrus, adversely affects the quality of the ovulatory follicle. Data from this study clearly indicate that it does – synchrony of oestrus comes at a cost, notably a reduction in the quality of the ovulatory follicle and hence the ability of the ewe to conceive.

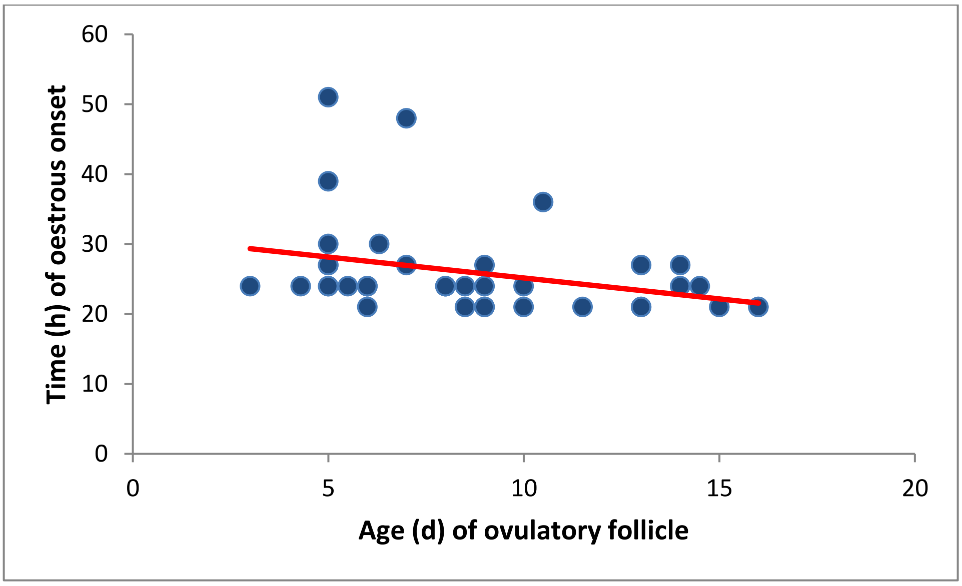
A major determinant of the quality of the ovulatory follicle is its age (i.e. when it first emerges). Emergence is largely determined by when the corpus luteum (CL) from the previous cycle regresses as indicated in Figure 2. As regression occurs, so the progesterone level falls, allowing ovulatory follicles to grow. The important point is that ovulatory follicles can emerge at any time during pessary treatment such that, at pessary removal, they range in age from 1 to 14 days.

**Figure 2. Relationship between the day of corpus luteum (CL) regression during pessary treatment and the day of emergence of the ovulatory follicle.**




In addition, a significant negative relationship was found between the age of the ovulatory follicle and time of onset of oestrus (Figure 3). Ewes with older ovulatory follicles came into oestrus earlier and with reduced variability whereas ewes with younger ovulatory follicles (i.e. those that emerge late in the pessary period) came into oestrus later and with greater variability. However, this relationship was influenced by season - in late Spring, the opposite occurred (i.e. older ovulatory follicles were associated with a later and more variable onset of oestrus).

**Figure 3. Relationship between age of the ovulatory follicle and time of onset of oestrus following pessary removal.** Data were collected in Autumn.



***Synopsis***. Treating ewes in Autumn with progesterone pessaries results in the age of ovulatory follicles varying widely ( 1 to 14 days) at pessary removal. Ewes with older follicles come into oestrus earlier and with less variability compared with ewes with younger ovulatory follicles. However, in late Spring, the opposite occurs. These results highlight the inability of the progesterone pessary to adequately control the development of the ovulatory follicle.

**4. Association between the age of the ovulatory follicle and pregnancy rate**

There is also a relationship between age of the ovulatory follicle and pregnancy rate (Table 1). Ewes in which ovulatory follicles emerge between Days 7-9 of the pessary period are more fertile than ewes in which emergence occurs at other times. The least fertile ewes are those with younger ovulatory follicles (i.e. those that emerged on or after Day 10). These results are relevant to cycling ewes and similar results may not be obtained in non-cycling ewes (e.g. in Spring). This information provides an important clue to the development of an improved protocol – controlling the time of emergence of the ovulatory follicle should lead to improved pregnancy rates.

The other interesting observation is the way in which pessary treatment increases the number of ewes in which ovulatory follicles emerge during the least fertile period of pessary treatment (Table 1). This is another unwanted side effect of pessary treatment. Hence, whilst pessary treatment is essential for synchrony of oestrus, it reduces ewe fertility by (1) having a direct effect on follicle quality and by (2) changing the distribution of ewes based on the time of emergence of the ovulatory follicle.

**Table 1. Percentage of pregnant ewes and litter size in ewes in which ovulatory follicles emerged at different times during pessary treatment ( Day 1 = day of pessary insertion).** Ewes were inseminated with chilled semen commencing 44h after pessary removal.

| Day of follicle emergence | Actual no. ewes | Expected no. ewes | Ewes pregnant (%) | Litter size (fetuses/pregnancy) |
|---|---|---|---|---|
| 1 – 6 | 45 | 110 | 77.8a[b] | 1.51 |
| 7 – 9 | 50 | 55 | 90.2[b] | 1.28 |
| 10 – 12 | 48 | 55 | 68.8[a] | 1.36 |
| ≥13 | 132 | 55 | 71.2[a] | 1.41 |

[a,b] Figures within column with a different superscript differ significantly

***Synopsis***. Ovulatory follicles that emerge during Days 7 – 9 of the pessary period are associated with a higher pregnancy rate than when emergence occurs at other times. This result is not due to an improvement in the synchrony of oestrus but to an improvement in follicle/oocyte quality. This result indicates that treatment protocols that control the time of emergence of the ovulatory follicle are required.

**DEVELOPMENT OF IMPROVED TREATMENT PROTOCOLS**

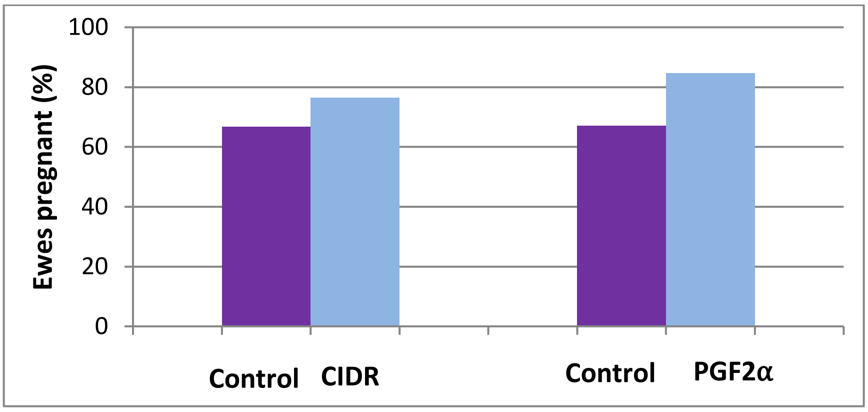
Two broad strategies to improve the synchrony of oestrus have emerged from these studies. They are (1) controlling the time of emergence of the ovulatory follicle and (2) improving the quality of young ovulatory follicles (i.e. those that emerge late in the pessary period).

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

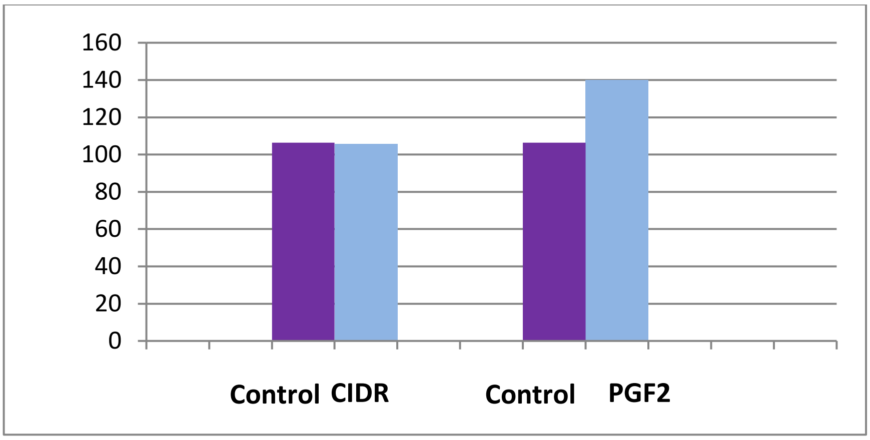
Emergence of the ovulatory follicle can be regulated by controlling when the CL regresses during the pessary period. The two options examined were (1) pre-treatment with an initial CIDR 21 days before the second CIDR is inserted and (2) pre-treatment with PGF2α 27 days before CIDR insertion. Both options have the potential to increase the likelihood of ovulatory follicles emerging during the preferred Days 7 – 9 period of pessary treatment. Both pre-treatments increased pregnancy rates (Figure 4a) but only the PGF2α pre-treatment increased the number of fetuses per 100 ewes (Figure 4b). This increase was 33.9%.

**Figure 4. The effect of pre-treatment with either a CIDR or PGF2α on AI outcomes using frozen-thawed semen**.

**(a) Pregnancy rate**




**(b) Fetuses/100 ewes inseminated**




With both treatments there were indications of an improved synchrony but these improvements were not significant. This result indicates that the higher pregnancy rate resulted from an improvement in the quality of the ovulatory follicle/oocyte.

## 2 Improving the quality of young ovulatory follicles

One side effect of pessary treatment is a disproportionate number of ewes with young ovulatory follicles at pessary removal (i.e. those that emerge late in the pessary period). In late Summer/Autumn, their presence results in a delayed onset of oestrus although this is not the case in late Spring. Early treatment with PMSG has the potential to improve the maturation of these follicles. Treatment 12h before pessary removal improved synchrony of oestrus (Figure 5) although the total ewes in oestrus was not affected (range 86.2 – 89.2%). Both pregnancy rate and litter size were increased by this treatment but the increases were not statistically significant (Figures 6).

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

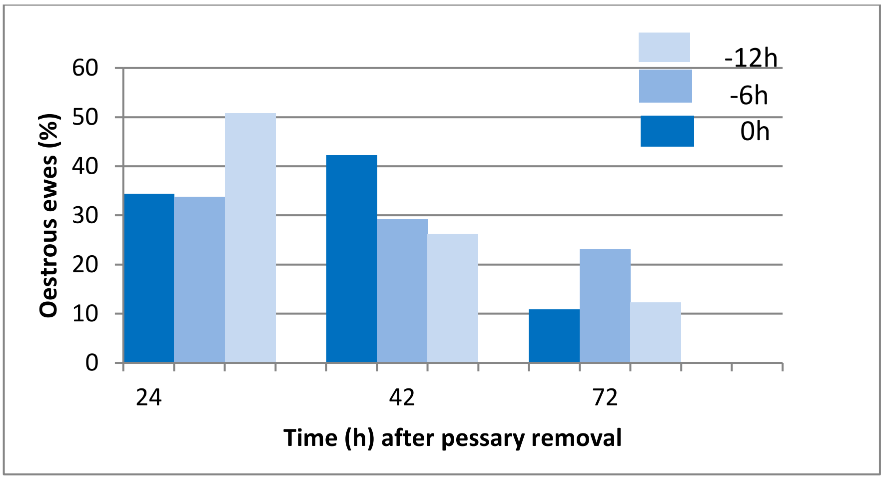
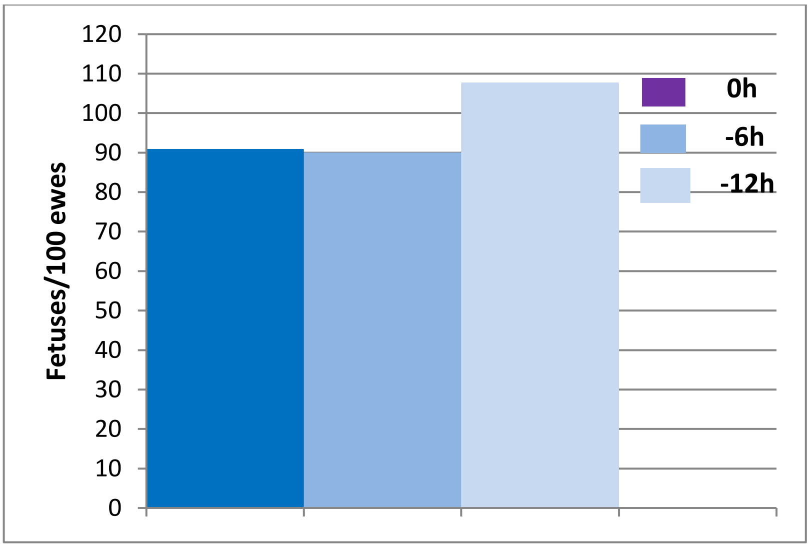



**Figure 6. Effect of PMSG treatment at 0h, -6h and -12h relative to pessary removal (0h) on the number of fetuses per 100 ewes inseminated** (ewes were inseminated 43h after pessary removal with frozen-thawed semen).

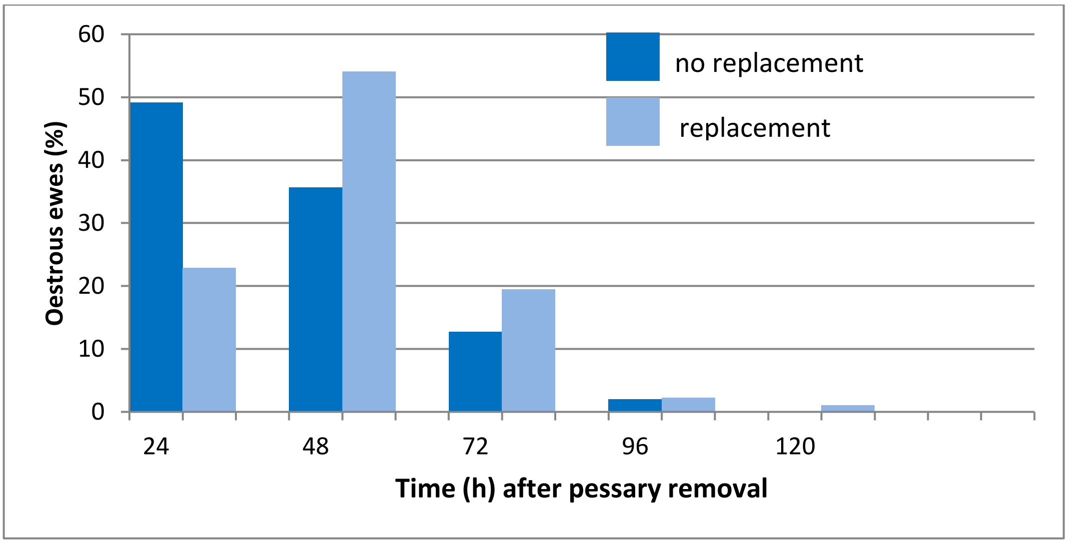


**3 Pessary replacement on Day 9**

A single pessary is unable to maintain the circulating levels of progesterone that are required to prevent early emergence of ovulatory follicles. In an overseas study where pessaries were replaced every 4 - 5 days (i.e. three pessaries were used), the early emergence of ovulatory follicles was constrained. The problem with a single pessary is not that it contains inadequate progesterone but that it becomes coated with a substance (presumably a mucopolysaccharide) that prevents its release.

Pessary replacement on Day 9 produced a significant delay in the pattern of oestrus (Figure 7) but, more importantly, the synchrony was improved. Further research is required to determine the importance of maintaining elevated progesterone levels, especially late in the pessary period. Pessary replacement may be useful in flocks with a poor AI history or in undernourished flocks given that these flocks experience a delay in the onset of oestrus (see below).

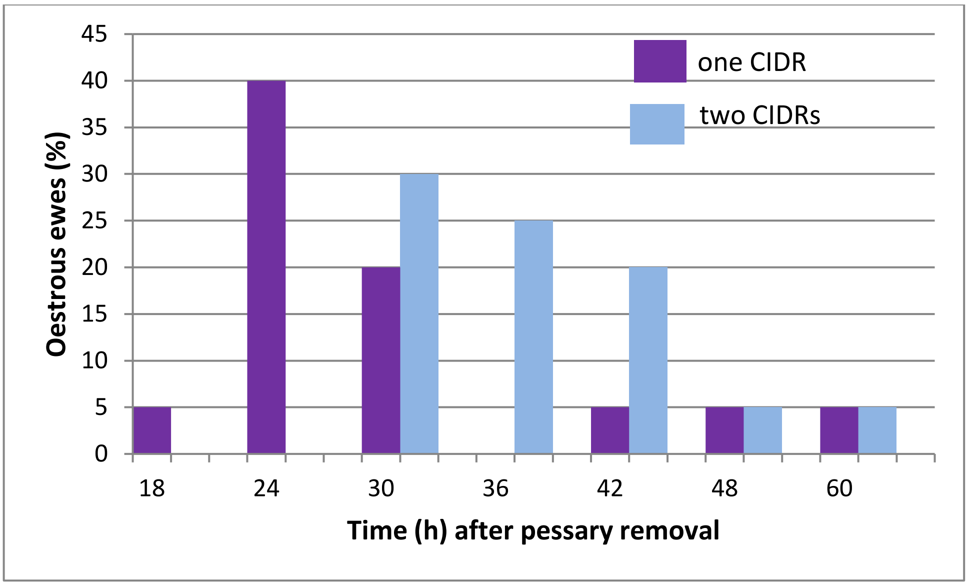
**Figure 7. Effect of pessary replacement (on Day 9) on the timing of oestrus.**




**4 One pessary versus two concurrent pessaries**

Given the low progesterone levels during pessary treatment, observations were made to determine if the use of two concurrent pessaries from Day 1 influenced the timing of oestrus and AI outcomes. Both treatments resulted in good patterns of oestrus (Figure 8). Not surprisingly, oestrus occurred earlier with one CIDR. Pregnancy rate and litter size were not affected.

**Figure 8. Effect of one or two concurrent pessaries on time of onset of oestrus following pessary removal**




***Synopsis.*** Controlling the time of emergence of the ovulatory follicle using PGF2α is a promising treatment for the improvement of AI outcomes. It operates by improving the quality of ovulating follicles rather than by improving the pattern of oestrus. It is currently being evaluated in commercial flocks. Early treatment with PMSG aimed to improve the maturation of young ovulatory follicles. Treatment improved the pattern of oestrus but AI outcomes were not improved, possibly due to inappropriate timing of insemination. Both treatments depend on ewes cycling naturally at pessary insertion. Observations on the use of multiple pessaries from Day 1 or their replacement on Day 9 indicate that the patterns of oestrus can be altered and possibly improved. There is a need to develop an improved pessary that is better able to maintain progesterone levels at desired levels.

**MANAGEMENT STRATEGIES TO IMPROVE AI SUCCESS RATES**

There are several strategies to improve AI outcomes.

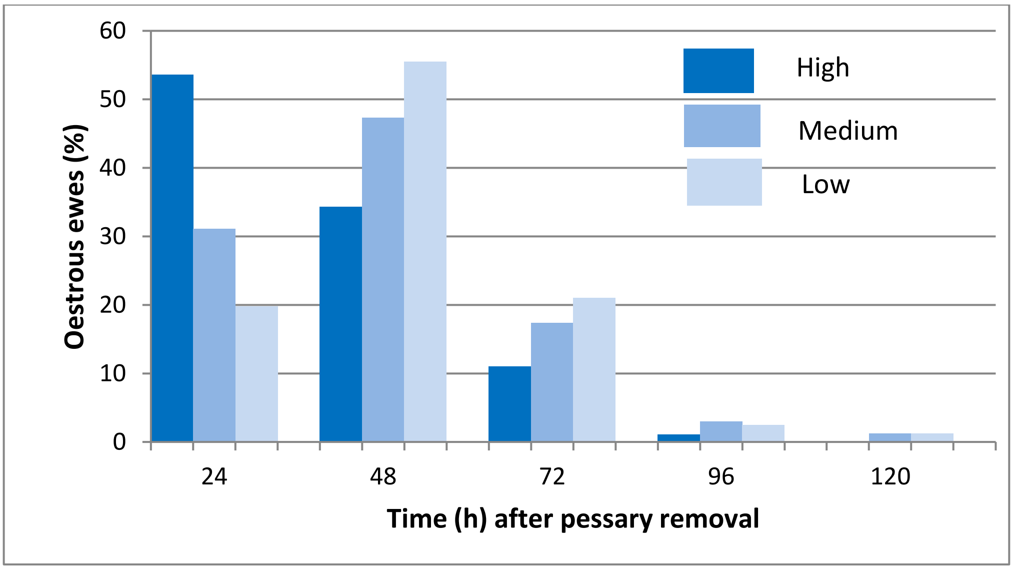
**1 Long and short term nutrition**

It takes approximately six months for follicles to fully mature before ovulating 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 were:

- Oestrus occurs earlier and is more synchronous (Figure 9) – hence insemination should commence occur earlier in these flocks.
- More ewes come into oestrus (91.9% versus 85.2% and 85.7% respectively).
- Pregnancy rates are higher (81.1% versus 71.1% and 73.7% respectively using chilled semen).
- Litter size is higher (1.50 versus 1.35 and 1.28 respectively).

On the other hand, high nutrition (1.75M) during the pessary period – **short term nutrition** - increased pregnancy rate (79.0% versus 72.3% for the 1.0M group) but there was no effect on either time of oestrus or litter size. The reasons for the higher pregnancy rate are not known but presumably they relate to improvements in oocyte quality during the final stages of maturation.

**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 during late Summer and Autumn, these results indicate the importance of nutrition during the months leading up to and during the pessary period. Importantly, an assessment of condition score at pessary insertion should give an indication of the optimal time to inseminate.

## 2 Using teaser marks

Teaser marks are valuable in AI programs. They give information on the normality of the pattern of oestrus and, hence, the pattern of ovulation.

### (a) Timing of AI

A major challenge in AI programs is when best to commence insemination. It has long been assumed that a standard time can be used and this is usually set at 48h after pessary removal. This is based on the sponge pessary but ewes treated with CIDRs ovulate about 6h earlier. Consequently, ewes with CIDRs should be inseminated earlier (e.g. from about 42h after pessary removal). However, this situation is complicated by other factors (e.g. nutrition, time of year, cyclicity) advancing or delaying the onset of oestrus. It is for this reason that teaser marks are valuable in characterising the normality of the synchrony.

Until variability in the patterns of oestrus can be controlled, one strategy is to commence insemination at a time indicated by the first occurrence of oestrus. With CIDRs, the first ewes in oestrus are usually observed in the 18 -24h period after pessary removal – a significant number of these ewes will ovulate from about 42 - 44h. A later onset (e.g. from 30h), necessitates a later insemination. Having the flexibility to either advance or delay timing to better match the pattern of oestrus is likely to improve pregnancy rates. However, there are numerous logistical constraints to this strategy and there is often no flexibility, particularly in large programs.

### (b) Inseminate in approximate order of occurrence of oestrus

The ability to inseminate ewes in this order is a strategy that helps overcome problems associated with incorrect timing of AI. This is particularly important in large programs where insemination occurs throughout the day. In this case, ewes inseminated late in the day may have ovulated many hours beforehand resulting in the presence of aged eggs. Whilst drafting ewes on the day of AI needs to be kept to a minimum, identification of ewes within early, late and intermediate categories based on the occurrence of oestrus will better align times of insemination and ovulation.

### (c) Not inseminating unmarked ewes

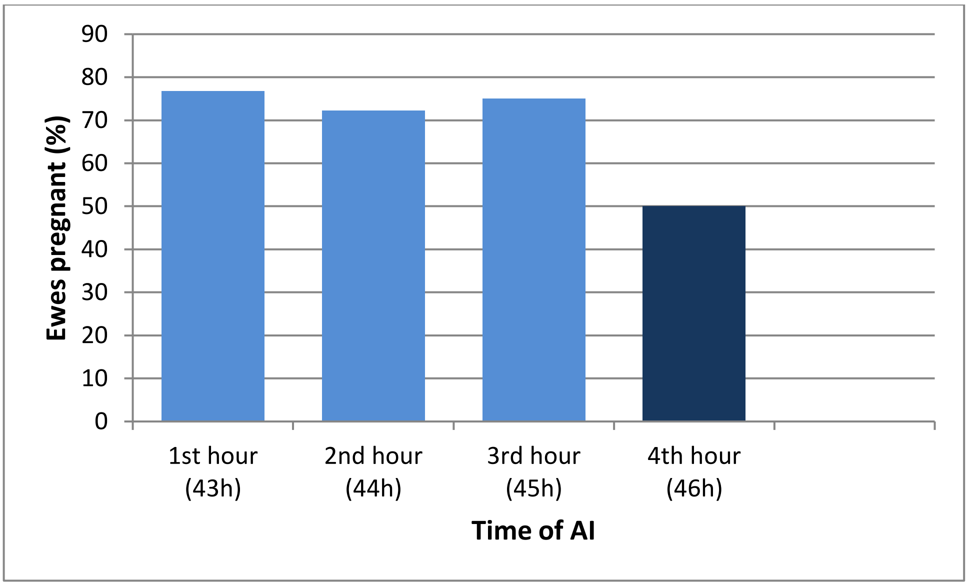
In most AI programs up to 20% of ewes are not marked by teasers at the time of insemination. This doesn't mean that these ewes won't ovulate and conceive. However, their insemination usually results in a reduced pregnancy rate and a decision to inseminate should be based on the value of lambs expected given the costs involved.

## 3 Managing ewes on the day of AI

Evidence indicates that pregnancy rates can be higher in ewes that are inseminated earlier in the day. This is demonstrated in data presented in Figure 10 where pregnancy rates in two separate four-hour programs declined during the last hour. It is not known if further reductions would occur after this time in longer programs. It is not known why such reductions occur but one possibility is that ova rapidly lose their ability to fertilise following ovulation.

Whatever the reason, this finding highlights the importance of staggering pessary removal (e.g. a.m. and p.m. groups) in large programs so that the times of insemination and ovulation are better matched.

**Figure 10. Effect of time of insemination on pregnancy rate.** Data are combined for two four-hour AI programs (insemination with frozen-thawed semen commenced at the start of the first hour, approximately 42h after pessary removal).




## 4 Other observations of interest

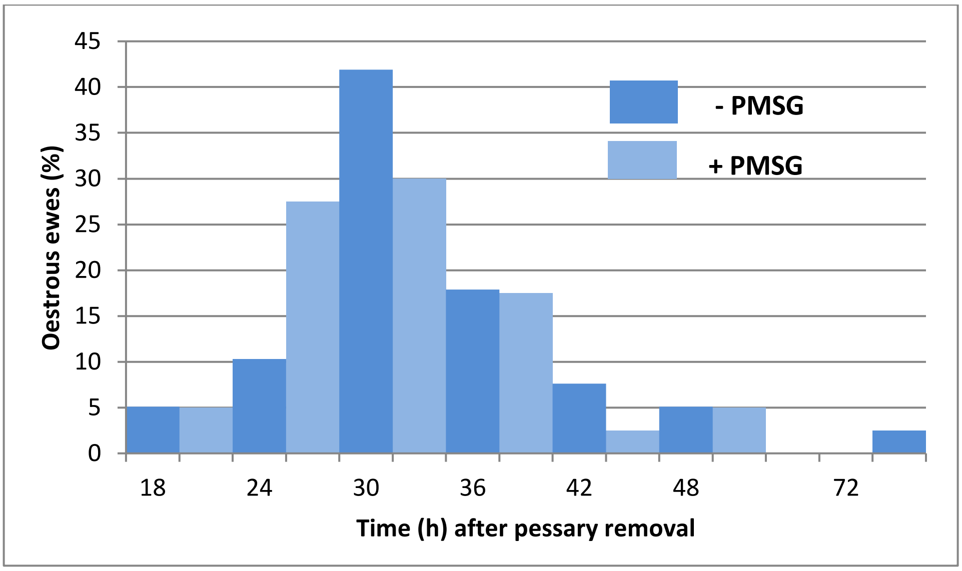
### (a) Dose of PMSG

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. There is no guaranty that similar results would be obtained at other times of the year.

### (b) 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 respectively were detected in oestrus. Despite this, the use of PMSG is recommended because of its likely benefit in situations where follicle growth is compromised (e.g. poor nutrition, heat stress). However, should PMSG not be available, it is still possible to conduct an AI program but it is recommended that insemination be based on the occurrence of oestrus.

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




**(c) 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. It is speculated that this "pseudo-oestrus" results from pessary treatment adversely affecting follicle development such that there are deficiencies in the pheromonal cascade necessary for stimulation. It is estimated that approximately 5 – 7% of ewes experience a "pseudo-oestrus" following pessary treatment.

**(d) Assessing the normality of oestrus by observing the uterus/ovaries**

The turgid nature of uterine horns is used as an indicator of the adequacy of the synchrony. However, turgidity occurs within the first 24h after pessary removal and can't be used as an accurate indicator. The ovarian surface, with emphasis on follicle size and colour, is a far better indicator. It has also been observed that the tract rapidly becomes flaccid after ovulation and this is interpreted as a positive sign given that, with correct timing of insemination, some ewes would be expected to ovulate during the insemination period.

### (e) Comparison of teasers treated with testosterone for either two or four weeks

Wethers treated with testosterone for the conventional two week period often appear to 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.

## CHALLENGES OF CONDUCTING AI IN SPRING

It is estimated that approximately 50% of flocks in Australia are inseminated in Spring (mainly late October and November) when a significant percentage of the flock are in anoestrus. This percentage varies widely between years – e.g. during the four-year AWI study this figure varied from 12% to 88%. The reasons for this are not known but it presents challenges when trying to develop improved treatment protocols for this time of year. Unique features of flocks prepared for AI in Spring include:

- The most fertile ewes are those that are in seasonal anoestrus at pessary insertion.
- These non-cycling ewes come into oestrus earlier than cycling ewes making an overall preferred time of insemination difficult.
- Insertion of the pessary causes some ewes 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.
- Variation in the age of the ovulatory follicle remains important but it is the younger follicles that are preferred – these are associated with an improved synchrony (opposite to the situation in Autumn).
- Fortuitously, one side effect of pessary treatment is an increase in the proportion of the flock with young ovulatory follicles and this improves the synchrony (in contrast with Autumn).
- Nutrition is usually good resulting in ewes generally coming into oestrus earlier than during late Summer or Autumn.

The new protocol (PGF2α pre-treatment) that improves AI outcomes in Autumn requires ewes to be cycling and so will be far less effective in Spring. Additionally, the use of early PMSG treatment to improve synchrony is based on young ovulatory being less competent than older follicles - but this is only the case in Autumn. More research is required to better understand how best to develop improved protocols for Spring. This research is planned for 2022/23.

**WHY HAVE AI RESULTS OFTEN BEEN DISAPPOINTING?**

Multiple factors are responsible for the poor results and the magnitude of disappointment is determined by how many factors are at play at any one time. The following are the major factors responsible.

**1 The pessary is inadequate**

The pessary (CIDR or sponge), whilst essential for synchrony of oestrus, is single-handedly responsible for a reduction in pregnancy rates. The conventional view is that pessary treatment prevents follicle growth and, on its removal, an ovulatory follicle matures and ovulates. This is far from what actually happens. The pessary does not prevent ovulatory follicles from emerging and growing. After emergence, these follicles are neither able to ovulate (because of the pessary) nor regress and, as a consequence, vary widely in age at pessary removal. Age of the ovulatory follicle affects pregnancy rates. The extent of any reduction in pregnancy rate is entirely up to chance – you may be lucky (lots of ewes with ovulatory follicles emerging during the preferred Day 7 – 9 period) or unlucky (lots of ewes with follicles that are either too old or too young). In addition, the pessary has the ability to increase the number of ewes with young ovulatory follicles – this is bad in Autumn (they are of poorer fertility) but probably good in Spring (they are off improved fertility).

**2 The pattern of oestrus is delayed or too variable**

A major failing of the AI industry (including producers, practitioners and researchers) has been the belief that pessary treatment provides an adequate synchrony. It does provide a synchrony but the important question is whether the synchrony is adequate to facilitate good pregnancy rates? One factor that has been identified as being a major determinant of the quality of the synchrony is the level of nutrition between the previous lambing/lactation and the cycle of AI (high nutrition gives an earlier onset of oestrus). There are management strategies to overcome this problem (e.g. inseminate ewes in batches as they are marked by teasers) but the best option is a treatment protocol that reliably generates a good synchrony. Pivotal to this challenge is the development of an improved pessary that is able to maintain adequate circulating levels of progesterone.

**3 Wrong time of insemination**

Because variability in the pattern of oestrus reflects the pattern of ovulation, timing of insemination to accommodate this variability is difficult. Consequently, it is very easy to inseminate at a less than optimal time. Factors that can affect the onset of oestrus include time of year, nutrition and whether or not ewes are cycling at pessary insertion. Despite this, a standard time of insemination is usually used. This situation is further confounded by this standard time being based on the use of the sponge pessary when insemination 48h after pessary removal was conventional. However, ewes treated with CIDRs ovulate 6h earlier than if treated with sponges. Ideally, the time of insemination is determined by when the first ewes come into oestrus but the ability to modify programs at the last moment is very limited.

**4 Semen quality**

It has long been assumed that poor semen quality is primarily responsible for poor pregnancy rates following AI. However, if a good synchrony is obtained, semen quality plays a relatively minor role – a good synchrony will give a good pregnancy rate provided the time of insemination is appropriate. Obviously, every effort needs to be made to use the best semen available but improvements in AI outcomes will depend more on the development of improved protocols for synchrony of oestrus than on any other factor.

**DEVELOPMENT OF IMPROVED TREATMENT PROTOCOLS**

This research has identified two strategies for the development of improved protocols. The protocol involving pre-treatment with PGF2α is the most promising resulting in a 33.9% increase in the number of fetuses per 100 ewes inseminated. A second protocol involves early treatment with PMSG 12h to improve the synchrony of ewes with young ovulatory follicles. This treatment improved the synchrony of oestrus but pregnancy rates were not improved (possibly due to incorrect timing of insemination). One constraint is that both of these options rely on cycling ewes and the extent to which they are useful in Spring remains to be determined. This issue will be subject to further research.

- **Conventional protocol** (single pessary and PMSG at pessary removal)
  - Can produce good results but is unreliable due to large variation in the pattern of oestrus 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 a later insemination.

- **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 but nicely synchronised oestrus.
  - Insemination from about 48h is required.
  - Whilst further research is required, this protocol might suit flocks with a poor AI history or undernourished flocks.

- **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.
  - Likely to improve the outcome in flocks with a history of poor AI performance, particularly those that experienced poor/delayed onset of oestrus.
  - The efficacy of this treatment in Spring is not known.
  - The protocol is unwieldy requiring PMSG to be given at difficult times (e.g. mid-night).

- **Pre-treatment with PGF2α**
  - This option is based on the conventional protocol but with PGF2α being administered 27 days before pessary insertion.
  - Treatment at this time increases the likelihood that ovulatory follicles will emerge during the preferred Days 7 – 9 of the pessary period.
  - This protocol is associated with significant improvements in pregnancy rate and the number of fetuses per 100 ewes inseminated.
  - Insemination at 42h after pessary removal is recommended.
  - This protocol is yet to be adapted for used in Spring.

- **PGF2α treatment immediately preceding a 7-day pessary period**

- This protocol has not been examined in this project but is used overseas.
- Treatment improves the growth of the ovulatory follicle.
- The likely preferred time of insemination is 48h after pessary removal.

**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<br>Insert CIDRs | |
| | | |
| 15 | Inject wethers – 2ml Ropel | |
| | | |
| | | |
| | | |
| 22 | Inject wethers – 2ml Ropel | |
| 23 | | |
| 24 | | |
| 25 | | |
| 26 | | |
| 27 | | |
| 28 | | |
| 29<br>**2 p.m**. | Inject wethers (2ml Ropel) and harness.<br><br>Remove CIDRs, inject 500 i.u. PMSG.<br>Run ewes and teasers together from CIDR removal. | Instead of harnesses, paint brisket with branding fluid.<br>To avoid marks from initial activity, consider delaying harnessing/painting for several hours after exposure.<br>Stagger CIDR removal by 3h in large programs. |
| 30<br>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<br>**8 a.m.** | Commence AI (42h after pessary removal).<br>Record oestrous marks at 2 p.m. (optional) | Option of drafting off marked ewes and inseminating first.<br>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 Table2. Treatment protocol for an AI program in which ewes are pre-treated with PGF2α.**

| 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<br>Inject wethers (2ml Ropel) | |
| 28 | | |
| 29 | | |
| 32 | | |
| 33 | | |
| 34 | Inject wethers (2ml Ropel) | |
| | | |
| 38 | | |
| 39 | | |
| 40<br>**2p.m.** | Inject wethers (2ml Ropel) and harness.<br><br>Remove CIDRs, inject 500 i.u. PMSG.<br>Run ewes with teasers from CIDR removal | Instead of harnesses, paint brisket with branding fluid.<br>To avoid marks from initial activity, consider delaying harnessing/painting for several hours after exposure.<br>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.<br>Consider delaying insemination (if possible) should oestrus be delayed |
| 42<br>**8a.m.** | Commence AI (usually 42h after pessary removal).<br>Record oestrous marks at 2 p.m. (optional) | Option of drafting off marked ewes and inseminating first.<br>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).