Improving the success of sheep artificial insemination programs

A handbook for practitioners

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SUMMARY

Research outlined in this document aimed (1) to understand why pregnancy rates can be disappointing following fixed-time AI and (2) to develop improved treatment protocols for synchrony of oestrus. Two factors were found to be responsible for poor pregnancy rates. Firstly, pessary treatment fails to control the time of emergence of the ovulatory follicle resulting in follicles ranging in age from 1 – 14 days at pessary removal. Pregnancy rates are highest (≥90% using chilled semen) when emergence occurs between Days 7 – 9 of the pessary period compared with 70 – 75% when emergence occurs at other times. In addition, older ovulatory follicles are associated with an improved synchrony in autumn but, in late spring, the opposite occurs with younger ovulatory follicles being associated with a better synchrony. The second factor is the inability of pessary treatment to induce a reliable synchrony due to the overriding effects of nutrition (both long and short-term), season and the cyclical status of the ewe. Consequently, timing of insemination is often less than optimal and this results in reduced pregnancy rates. To overcome these problems, modifications to the conventional treatment protocol have been examined including pessary replacement on Day 9, pre-treatment with PGF2α to control the time of follicle emergence, early treatment with eCG and the concurrent use of two pessaries. Pessary replacement delays the onset of oestrus but improves synchrony resulting in improved pregnancy rates. Replacement overcomes the effects of nutrition and season, resulting in a reliable synchrony that enables insemination to routinely occur 48h after pessary removal. The other three modifications all show promise but further research is required. Numerous flaws in the implementation and conduct of AI programs have been identified and the adoption of recommendations outlined in this document should improve AI success rates.

BACKGROUND INFORMATION

1. Introduction

This document outlines the findings of research conducted to improve the success of sheep AI programs. The research was based partly on the outcomes of a SASMBA survey of the 2011 and 2012 AI seasons. 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 poor results. These data indicated a need to re-evaluate the methodology of synchronisation (largely unchanged for 60 years) and to develop new strategies to improve AI success rates.

The research used trans-rectal ultrasonography to examine ovarian follicle activity during pessary treatment. From the data collected, new details on the relationships between follicle growth patterns, emergence of ovulatory follicles and reproductive outcomes following AI have 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) the development of improved treatment protocols for synchrony of oestrus and improved pregnancy outcomes.

2. Variations in patterns of oestrus

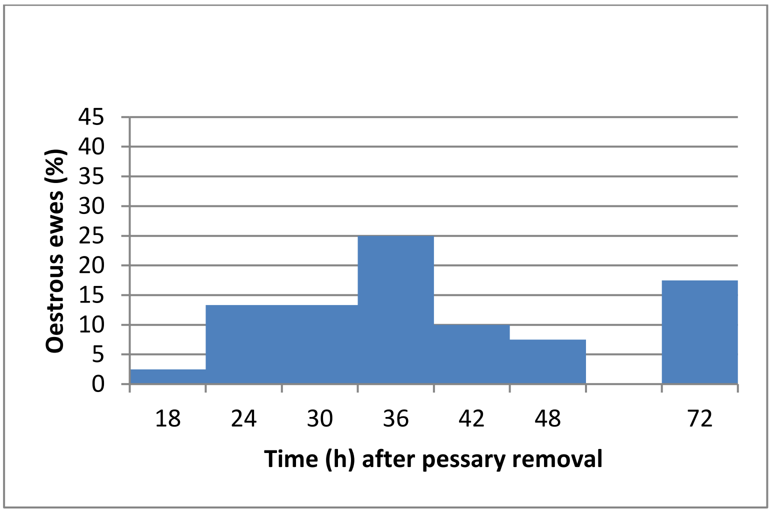
Variability in the onset of oestrus (and hence ovulation) is a primary cause of infertility in fixed-time AI programs. This variability makes it difficult to determine the optimal time of insemination which, historically, has been "fixed" but which has needed to be "flexible" to match the pattern of oestrus. With conventional 14-day pessary treatment, patterns of oestrus can range from very good to very poor (Figure 1a - e) raising the question of what is a normal oestrous? Patterns of oestrus can be assessed using the following criteria:

- Time when the first oestrous ewes are detected. Generally, a minimum of 10 – 20% of the flock should be in oestrus 24h after pessary removal although this figure can be as high as 40 - 60%. The absence of oestrous ewes at 24h is indicative of a delayed synchrony.
- The percentage of the flock not detected in oestrus at the commencement of insemination (which should occur from 42 - 44h after pessary removal with conventional treatment). Ewes that cycle after this time are of reduced fertility.
- The percentage of the flock that fails to come into oestrus (usually 10 – 20%). Overall, 30 – 40% of ewes not detected in oestrus conceive to AI.

Variability in the onset of oestrus within a flock is innate and due to differences in concentrations of oestrogen and progesterone around the time of pessary removal. However, there are much larger sources of variability due to the effects of nutrition, season and the cyclical status of the ewe and, as describe below, pessary treatment is unable to mitigate these effects.

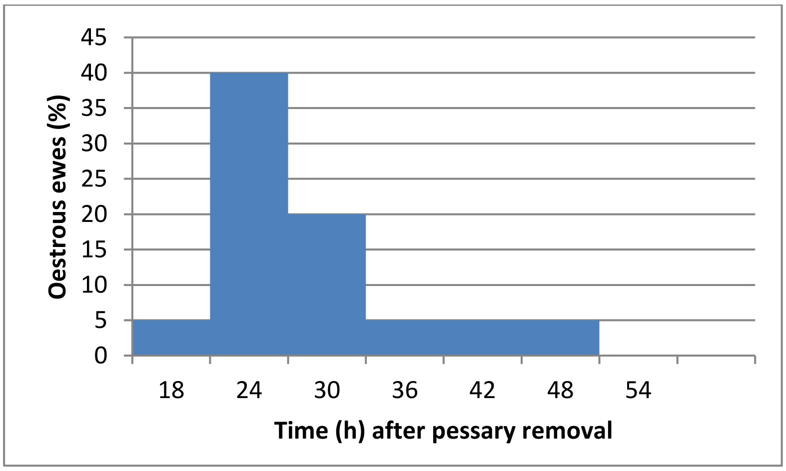
Figure 1. Examples of time of onset of oestrus following pessary removal. These charts plot the percentage of new ewes in oestrus (using teasers with harnesses and crayons) at 6-h intervals after pessary removal. Observations were conducted up to 48h after pessary removal with a final observation at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

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



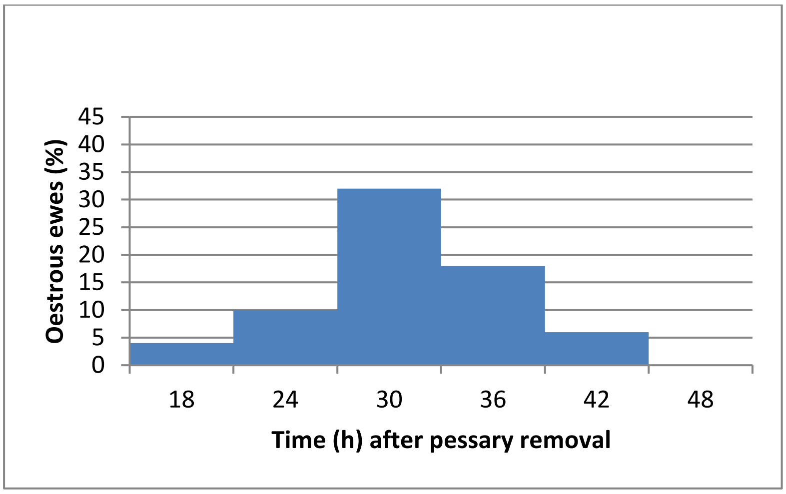
- 15.0% in oestrus at 24h
- 12.5% not in oestrus
- 17.5% in oestrus after 48h

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



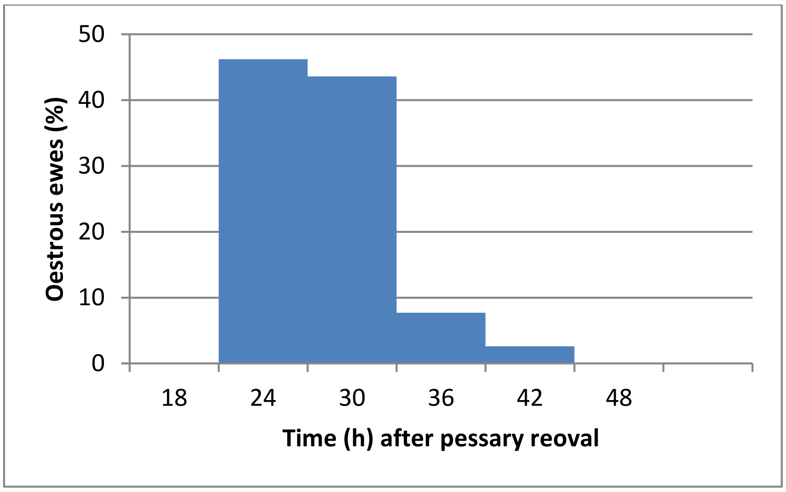
- 45.0% in oestrus at 24h
- 20.0% not in oestrus
- 0.0% in oestrus after 48h

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



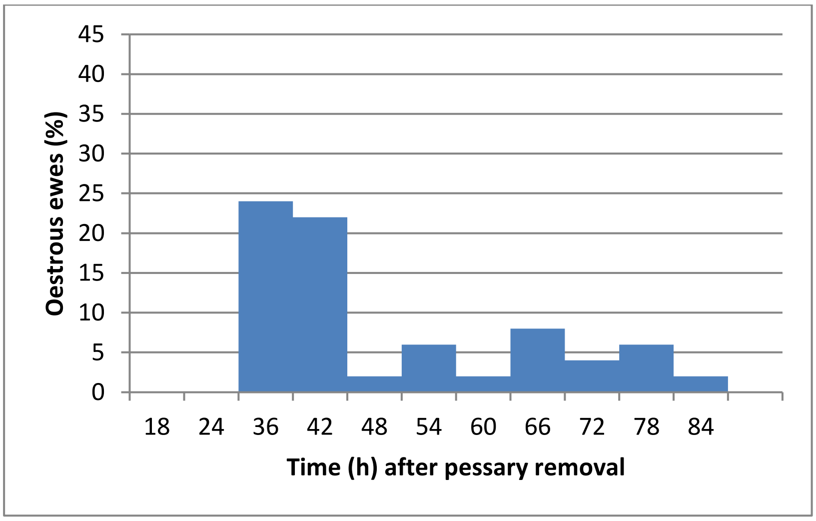
- 14.0% in oestrus at 24h
- 30.0% not in oestrus
- 0.0% in oestrus after 42h

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

CAUSES OF POOR PREGNANCY RATES IN AI PROGRAMS

Two major causes of infertility have been identified.

1. Pessary treatment affects quality of the ovulatory follicle

Whilst pessary treatment is essential for synchrony of oestrus, it comes at the cost of reduced follicle and oocyte quality. The pessary is unable to control the emergence of the ovulatory follicle which is determined by the time of luteal regression and, as a consequence, emergence occurs randomly during pessary treatment. As a result, ovulatory follicles vary in age from 1 – 14 days at pessary removal. This is important because the age of the ovulatory follicle affects both the timing of oestrus and pregnancy rate.

(a) Onset of oestrus

In autumn, ewes with older ovulatory follicles (i.e. those that emerge early) come into oestrus earlier and with reduced variability whereas ewes with younger ovulatory follicles (i.e. those that emerge late) come into oestrus later and with greater variability. However, this relationship is influenced by time of year. In spring (and early summer), the older ovulatory follicles are associated with a later and more variable onset of oestrus whilst the younger follicles are associated with an earlier and less variable onset. These differences are likely due to changes in the steroidogenic ability of ovulatory follicles.

(b) Pregnancy rate

Ewes in which ovulatory follicles emerge between Days 7 - 9 of the pessary period are the most fertile (Table 1). The least fertile ewes are those in which emergence occurs after Day 9 whilst ewes in which emergence occurs before Day 7 are of intermediate fertility. In randomly cycling flocks, the percentage of ewes that fall within the preferred Day 7-9 occurs by chance. This study was conducted in autumn and similar findings may not occur in spring, a time when many ewes are not cycling naturally. Never-the-less, these findings indicate the importance of developing a protocol that not only synchronises oestrus but also controls the time of emergence of the ovulatory follicle. This topic is addressed later.

Table 1. Pregnancy rates 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	No. ewes	Ewes pregnant (%)	Litter size (fetuses/pregnancy)
1 – 6	45	77.8a[b]	1.51
7 – 9	50	90.2[b]	1.28
10 – 12	48	68.8[a]	1.36
≥13	132	71.2[a]	1.41

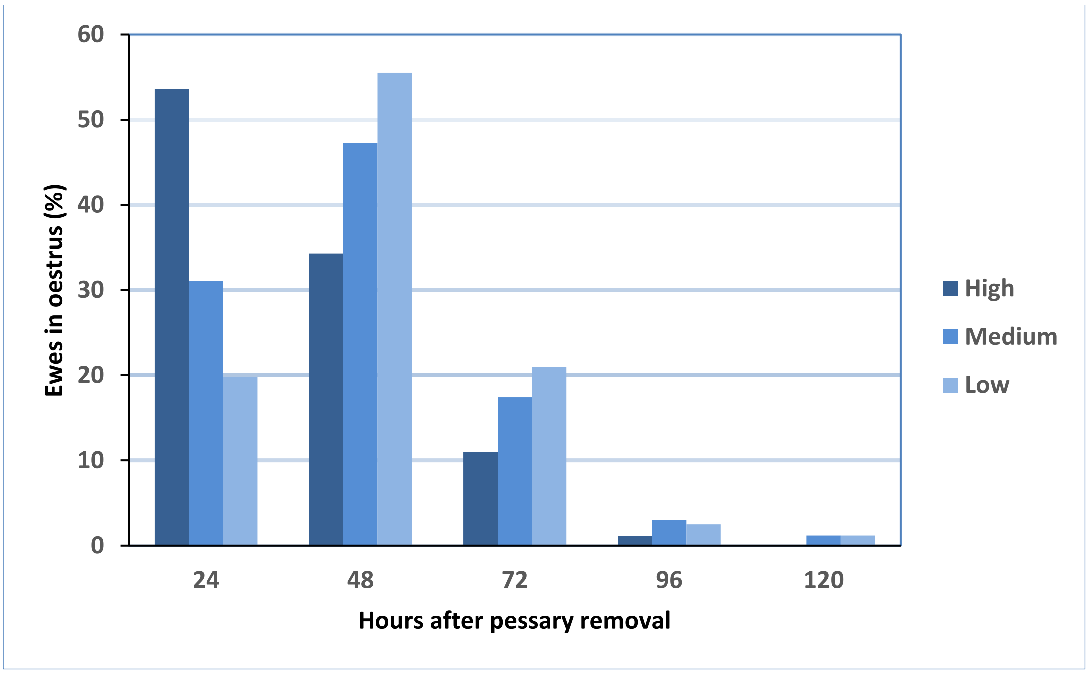
[a,b] Figures within column with a different superscript differ significantly

2 Environmental factors affect the ability of the pessary to synchronise oestrus

The more important cause of infertility is the inability of pessary treatment to produce a reliable and repeatable synchrony. Whilst there is innate variability in the pattern of oestrus (due to differences in steroid concentrations), there is also substantial variability due to the inability of pessary treatment to control the effects of nutrition, time of year and cyclical status of the ewe.

High long-term nutrition (from the previous lambing until the cycle of AI) can advance the onset of oestrus by up to 17h compared with medium or low levels (Figure 2). However, this difference is moderated (reduced to 6.4h or less) when nutrition during the pessary period (short-term nutrition) is increased from a maintenance (M) level to 1.5M. These results indicate that the nutritional status of the flock needs to be considered when planning the time of insemination. The effect of season is less severe with the onset of oestrus occurring up to 8 – 9h later in spring compared with autumn. This delay is due, at least partly, to the presence of non-cycling ewes which are later to cycle than are cycling ewes. In Merino flocks, the percentage of non-cycling ewes in spring varies widely between years and this is a further complication when planning AI programs.

Figure 2. 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 and the cycle of AI.



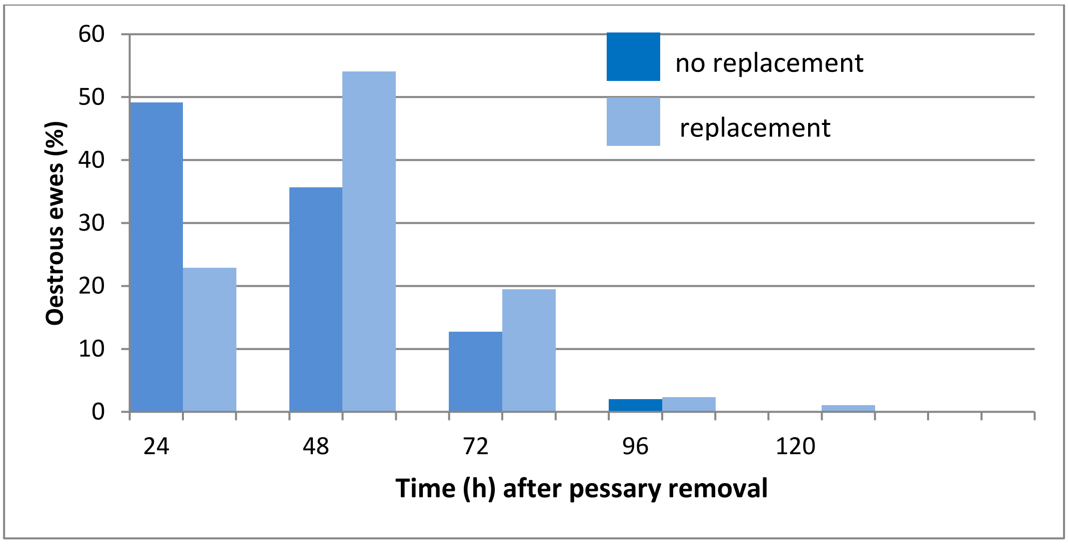
DEVELOPMENT OF IMPROVED TREATMENT PROTOCOLS

Several modifications to the conventional protocol have been investigated.

1. Pessary replacement on Day 9

Conventional pessary treatment provides inadequate progesterone during the last week of treatment. This deficiency adversely affects the maturation of ovulatory follicles and contributes to an unreliable onset of oestrus. Replacing pessaries on Day 9 results in a delayed but better synchrony (Figure 3). Importantly, replacement nullifies the effects of nutrition and probably season on synchrony and hence a more reliable synchrony results. This enables insemination to occur at a set time (48h after pessary removal) thus removing the uncertainty associated with variable patterns of oestrus. In our research, pregnancy rates (in good performing programs) are improved by approximately 10% when insemination commences 48h after pessary removal. It is recommended that pessary replacement becomes a standard procedure in the synchronisation protocol. The cost of replacing CIDRs can be offset by their re-use after cleaning and sterilising. A considerable amount of progesterone remains in used pessaries due to the development of a surface film that impedes diffusion

Figure 3. Effect of pessary replacement on Day 9 of pessary treatment on the time of onset of oestrus.



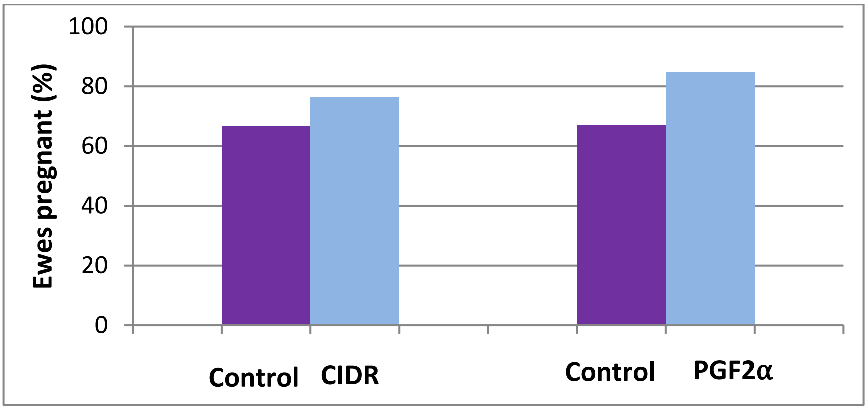
2. Controlling the time of emergence of the ovulatory follicle

Controlling the time of emergence of the ovulatory follicle is desirable because emergence between Days 7 – 9 is associated with improved pregnancy rates (Table 1). Emergence is not influenced by pessary treatment but controlled by luteal regression. However, there are two pre-treatments that are able to control time of emergence namely (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. In our studies, each pre-treatment increased pregnancy rate (Figure 4a) but only the PGF2α pre-treatment increased the number of fetuses per 100 ewes (Figure 4b). This increase was 33.9%. Other treatment options with PGF2α (e.g. treatment 12 days before pessary insertion and/or during the pessary period) were not beneficial.

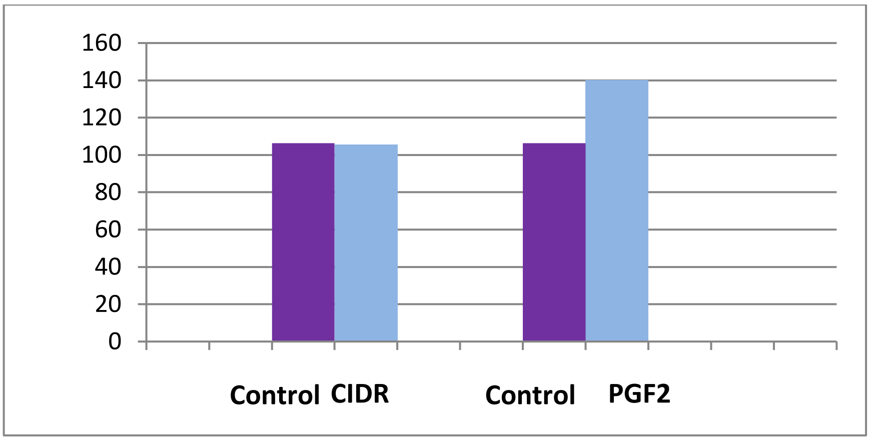
Neither pre-treatment altered the pattern of synchrony indicating that the higher pregnancy rates resulted from improvements in the quality of the ovulatory follicle/oocyte. However, the robustness of this protocol needs to be further examined before a recommendation on its value can be made. Additionally, this strategy requires ewes to be cycling naturally and consequently its use in spring is limited.

Figure 4. The effect of pre-treatment with either a CIDR or PGF2α on AI outcomes using frozen-thawed semen. Insemination commenced 43h after pessary removal.

(a) Pregnancy rate



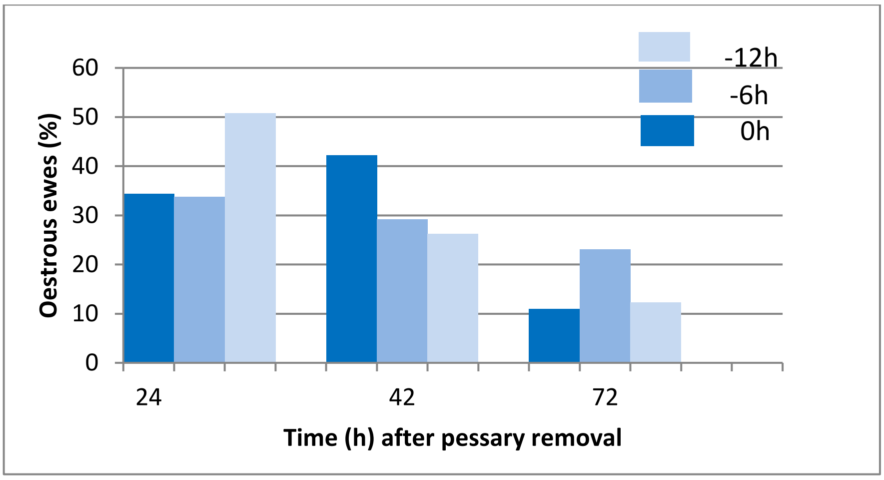
(b) Fetuses/100 ewes inseminated



3. Early treatment with eCG

Treatment with eCG usually occurs at pessary removal to stimulate follicle growth and to improve synchrony of oestrus. However, treatment 12h beforehand results in an earlier and better synchrony (Figure 5) necessitating an early insemination (from 42 – 43h after pessary removal). In our studies, the number of fetuses per 100 ewes inseminated was improved by 17%. However, further assessment of this protocol is required.

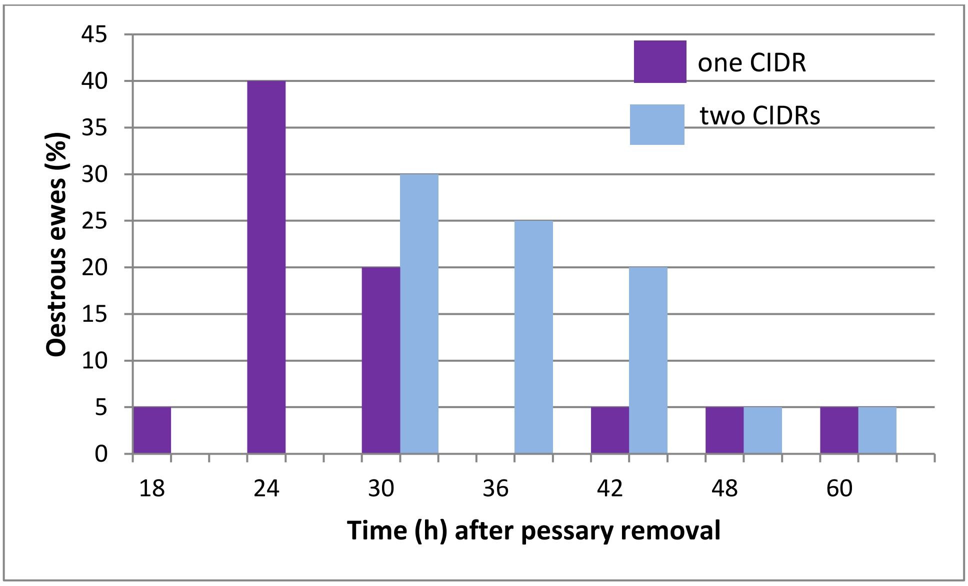
Figure 5. Effect of eCG treatment at 0h, -6h and -12h relative to pessary removal (0h) on time of onset of oestrus.



4 The use of two concurrent pessaries

Given that progesterone levels late in the pessary period are too low, observations were made to determine if the use of two concurrent pessaries (from Day 1) influences the onset of oestrus and pregnancy rates. Treatment resulted in a delayed but improved synchrony (Figure 6) but neither pregnancy rate nor litter size were improved. Failure to improve AI outcomes may have resulted from inappropriate timing of insemination (44h). It is likely, that with correct timing, this strategy could be a suitable alternative to replacing pessaries on Day 9. Further research is required.

Figure 6. Effect of one or two concurrent pessaries on time of onset of oestrus following pessary removal



MANAGEMENT STRATEGIES TO IMPROVE AI SUCCESS RATES

Several strategies are available.

1 Long and short-term nutrition

It takes approximately six months for follicles to fully mature and this corresponds with the interval between the previous lambing and the cycle of AI. As described above, nutrition during this period influences the success of AI programs. The benefits of high nutrition (BCS 4.0^{+}) compared with either medium (BCS 3.3^{+}) or low (BCS 2.7^{+}) nutrition include:

- An earlier and more synchronous oestrus (Figure 2).
- More ewes in oestrus (91.9% versus 85.2% and 85.7% respectively).
- Higher pregnancy rate (81.1% versus 71.1% and 73.7% respectively using chilled semen).
- Higher litter size (1.50 versus 1.35 and 1.28 respectively).

On the other hand, high nutrition (1.5M) during the pessary period increases pregnancy rate (79.0% versus 72.3% for the 1.0M group) but time of oestrus and litter size are not affected. The higher pregnancy rate is presumably related to improved oocyte quality. Importantly, a high diet during the pessary period (1.5M versus 1.0M) moderates the effects of long-term nutrition (i.e. the differences in the timing of oestrus due to long-term nutrition are reduced).

Because nutrition has a substantial effect on the timing of oestrus, insemination needs to be timed to match the overall BCS of the flock. It is estimated that the preferred times of insemination are 42h, 48h and 54h after pessary removal for flocks exposed to conditions of high, medium and low long-term nutrition (assuming a 1.0M diet during the pessary period). However, as described above, the need to match the insemination time with the BCS of the flock is eliminated when pessaries are replaced on Day 9.

2 Using teaser marks

Teaser marks provide information on the normality of the pattern of oestrus and indicate when insemination should commence.

(a) Timing of AI

A major challenge in AI programs is the optimal timing of insemination which is often “fixed” irrespective of the pattern of oestrus. In addition to the effects that nutrition, time of year and cyclicity have on the onset of oestrus (as outlined above), both pessary type (CIDR approximately 6h earlier than sponge) and ewe age (maidens approximately 6h earlier than older sheep) also affect the timing of oestrus. Given these variables, teaser marks are valuable in identifying the normality of the synchrony.

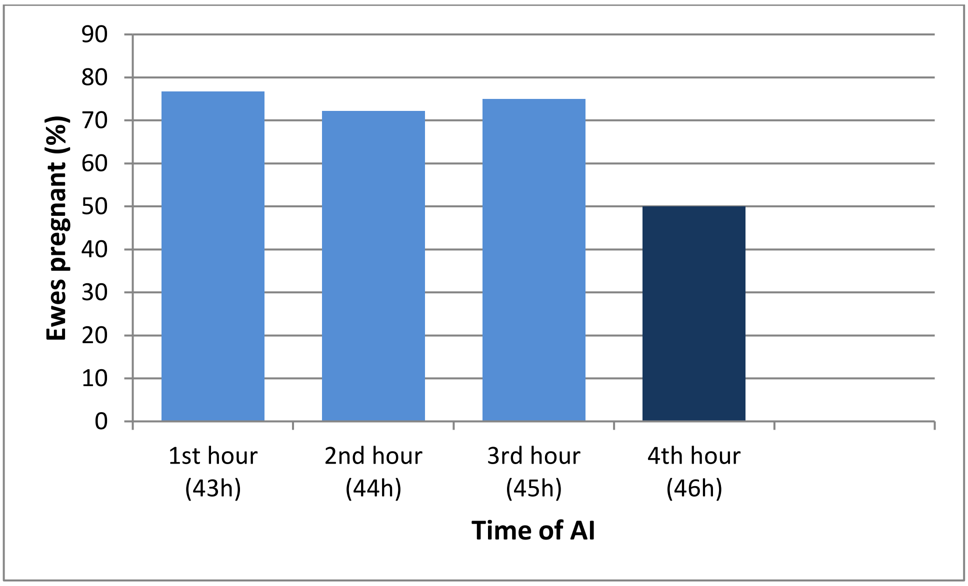
(b) Inseminate in approximate order of onset of oestrus

An insemination time based on the occurrence of oestrus helps overcome problems associated with poor synchrony. This is particularly important in large programs where ewes inseminated late in the day are likely to have ovulated numerous hours beforehand. This strategy better aligns the times of insemination and ovulation.

3 Managing ewes on the day of AI

Anecdotal evidence indicates that pregnancy rates are higher in ewes that are inseminated early in the day's program. This is also indicated in Figure 7 in which data from two of our programs are combined. Insemination occurred over a four-hour period and, in each case, pregnancy rates declined after the first three hours. The reasons are not known but it highlights the importance of staggering pessary removal in large programs to facilitate comparable morning and afternoon groups.

Figure 7. Effect of time of insemination on pregnancy rate. Data are combined from two insemination programs (each of 200 ewes). Insemination with frozen-thawed semen commenced at the start of the first hour, approximately 42h after pessary removal.



4 Other observations of interest

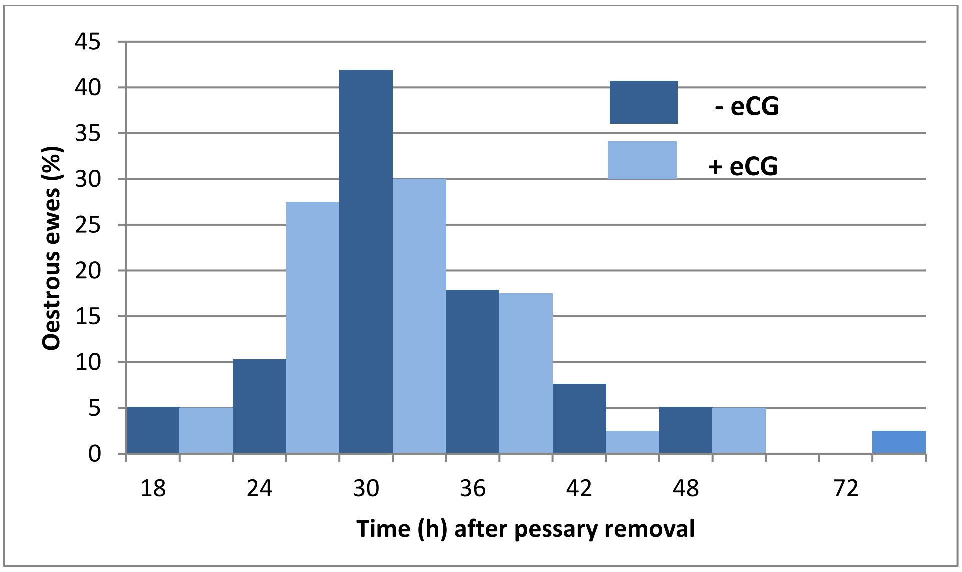
(a) Dose of eCG

eCG treatment is essential when anoestrous ewes are induced to cycle. In our observations in spring, a higher dose (500 i.u. versus 400 i.u.) significantly increased litter size (1.50 versus 1.13) and slightly more ewes were in oestrus at 24h (11.7% versus 4.0%). Overall, more ewes were marked by teasers (91.5% versus 86.1%, a non-significant difference) compared with ewes treated with 400i.u. Similar results may not be obtained at other times of the year.

(b) Synchrony of oestrus without eCG

Data presented in Figure 8 indicate that a good synchrony can be obtained in autumn without eCG treatment. Overall, 87.2% and 87.5% of the –eCG and +eCG groups respectively were detected in oestrus and there was no difference in the pattern of oestrus. Despite this, the use of eCG is recommended because of its likely benefit when follicle growth is compromised (e.g. poor nutrition, heat stress, seasonal anoestrus). Whilst this study has not been replicated, the result indicates that should eCG not be available, it is possible to conduct an AI program. However, a comparable result may not be obtained in spring.

Figure 8. Timing of oestrus after pessary removal in ewes treated or not treated with eCG (400 i.u.). This study was conducted in autumn.



(c) Occurrence of a "pseudo-oestrus"

Some ewes can display oestrous behaviour without attracting the attention of teasers. It is speculated that this "pseudo-oestrus" results from pessary treatment adversely affecting the pheromone cascade. It is estimated that approximately 5 – 7% of ewes experience a "pseudo-oestrus".

(d) Appearance of the uterus/ovaries

The turgid nature of uterine horns is used as an indicator of the well-being of the synchrony. However, turgidity occurs within the first 24h after pessary removal (as oestrogen levels rise) and is not a worthwhile indicator of a timely onset of oestrus. On the other hand, it has been observed that the tract rapidly becomes flaccid after ovulation (as oestrogen levels decline) and this can be a useful indicator of normality.

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

Wethers treated for either two or four weeks were compared with entire rams in their ability to detect oestrous ewes. There were no differences in the ability to detect oestrus although the longer treatment tended to improve teaser vigour.

CHALLENGES OF CONDUCTING AI IN SPRING

It is estimated that up to 75% of Merino flocks in Australia are inseminated in spring/early summer when many ewes are in anoestrus. The percentage of anoestrous ewes varies widely between years (e.g. 12 – 88% in November during the four-year AWI study). The reasons for this variability are not known but it presents challenges in AI programs. Unique features of AI in spring include:

- The most fertile ewes are those that are in anoestrus at pessary insertion.
- The non-cycling ewes come into oestrus later than cycling ewes.
- The pessary causes some ewes to undergo spontaneous luteinisation.
- These luteinised follicles produce progesterone and this should be beneficial.
- The younger ovulatory follicles (i.e. those that emerge late in the pessary period) are associated with an improved synchrony compared with older ovulatory follicles. This is opposite to the situation in Autumn.

In our studies, the onset of oestrus occurred 8 – 9 h later in spring than in autumn Delaying insemination in spring will accommodate the later onset of oestrus in non-cycling ewes but will be a disadvantage to naturally cycling ewes. Insemination 48 h after pessary removal is a good compromise – being a little late for naturally cycling ewes (42 – 43h preferred) and a little early for non- cycling ewes (53 – 54h preferred). As mentioned earlier, it is expected that pessary replacement on Day 9 will eliminate the difference between cycling and non-cycling ewes.

THE PREFERRED PROTOCOL

Currently the preferred protocol is the standard protocol (CIDR, eCG) in which pessaries are replaced on Day 9. This results in a delayed but more synchronous oestrus with insemination commencing 48h after pessary removal.

Appendix Table 1. The preferred protocol for synchrony of oestrus in which pessaries are replaced on Day 9.

Day	Activity	Comments
1	Inject wethers (n=10% of ewe no.) – 2ml Ropel	Ropel requires a weekly injection.
8	Inject wethers – 2ml Ropel **Insert CIDRs**	
15	Inject wethers – 2ml Ropel	
17	**Replace CIDRs**	Can re-use old CIDRs (washed and sterilised).
18		
19		
20		
21		
22 **8 a.m.**	Inject wethers (2ml Ropel) and harness. **Remove CIDRs**, inject 500 i.u. eCG. Run ewes and wethers together.	Alternatively, paint brisket with branding fluid. Stagger CIDR removal in large programs.
23	Record oestrous marks from 8 a.m. (alternatively observe teaser activity).	Activity indicates normality of synchrony.
27 **8 a.m.**	Commence AI (48h after pessary removal).	Option of drafting off marked ewes and inseminating first.

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