Improving the success of sheep artificial insemination programs

A handbook for producers

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SUMMARY

Poor synchrony of oestrus is the primary cause of disappointing AI results in sheep. In this document, background information on this problem is provided together with strategies to improve the success of AI programs. Research has identified that pessary treatment, whilst essential for synchrony of oestrus, adversely affects egg quality and, consequently, pregnancy rates. In addition, treatment fails to produce a reliable synchrony due to the overriding effects of nutrition (both long and short-term), season and whether ewes are cycling naturally. Due to the variable onset of oestrus, timing of insemination is often less than optimal and this is the major cause of reduced pregnancy rates. To overcome these problems, several modifications to the standard treatment protocol have been investigated. It is recommended that pessaries be replaced on Day 9 of the treatment period (using either new or used pessaries). This results in a more reliable synchrony and pregnancy rates are improved. A second promising modification involves pre-treatment with a prostaglandin to control the time of emergence of the ovulatory follicle. Significant increases in pregnancy rates have been obtained but on-farm evaluation is required. Management strategies that can assist in improving AI success rates are also outlined in this document.

BACKGROUND

AI success rates have, over many decades, 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 the most likely cause. Subsequently, research funded by AWI/SASIF was conducted to unravel the complexities of oestrous synchronisation and to develop improved protocols for synchrony of oestrus. This document provides a summary of these findings together with recommendations to improve the success of AI programs.

WHY AI RESULTS CAN BE DISAPPOINTING

Poor AI results are a combination of two main factors.

1. **Pessary treatment adversely affects egg quality**

Whilst pessary treatment is essential for synchrony of oestrus, it comes at the cost of reduced egg/embryo quality. During pessary treatment, ovulatory follicles emerge at any time and are between 1 – 14 days of age at pessary removal. It is now known that pregnant is more likely when emergence occurs between Days 7 – 9 of the pessary period. In our studies, pregnancy rates of

≥90% were obtained in these ewes compared with 70 – 75% when emergence occurred at other times (using chilled semen). The percentage of ewes in which ovulatory follicles emerge during the preferred period occurs by chance. This finding provides a key to improving pregnancy rates, namely the development of treatments that not only synchronise oestrus but also control the time of emergence of the ovulatory follicle. Progress on this approach is outlined later in this document.

2. Pessary treatment fails to produce a reliable synchrony

Poor and variable synchrony occurs both within and between flocks and this variability often leads to insemination times being less than optimal.

Most ewes within a flock come into oestrus over a 24 – 36h period. This variability is innate and results from differences in concentrations of oestrogen and progesterone. However, there are other more important sources of variability over which pessary treatment has no control. These sources include differences in nutrition, season and the cyclical status of the ewe. High long-term nutrition (from the previous lambing) can advance the onset of oestrus by up to 17h compared with lesser nutrition indicating just how easy it is to inseminate at the wrong time. Interestingly, this difference is moderated (reduced to 6.4h or less) when nutrition during the pessary period (short-term nutrition) is increased from a maintenance level (M) to 1.5M. These results indicate that nutrition (both long and short-term) needs to be considered when planning AI programs, particularly the time of insemination. The effects of season are 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 compared with naturally cycling ewes. These results indicate that the use of a standard time of insemination (e.g. 48h after pessary removal) throughout the year is inadequate.

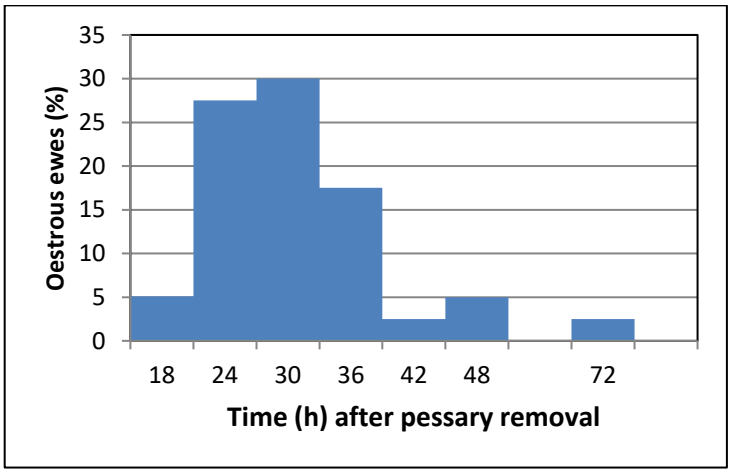
Ideally, insemination times are adjusted to match shifts in synchrony but late changes to these times are seldom possible. Never-the-less, it is important to assess the normality of the pattern of oestrus and this can be done using the following criteria:

- Time when ewes are first observed in oestrus (a minimum of 10 – 20% of ewes in oestrus at 24h).
- Percentage of the flock not detected in oestrus at the commencement of insemination (no more than 30 – 35%).
- Percentage of the flock that ultimately fails to be marked by teasers (up to 20 - 25%).

Examples of patterns of synchrony are shown in the figure below.

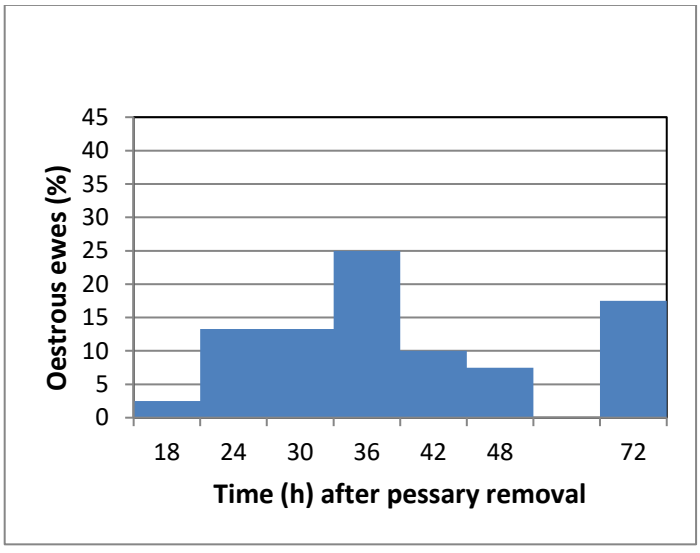
Figure 1. Example of a good (Flock 1) and a moderate synchrony (Flock 2) of oestrus. These charts plot the percentage of new ewes in oestrus (using teasers with harnesses and crayons) at 6-h intervals after pessary removal.

Flock 1 (good synchrony; total ewes in oestrus = 97.5%)



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

Flock 2 (moderate synchrony; total ewes in oestrus = 87.5%)



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

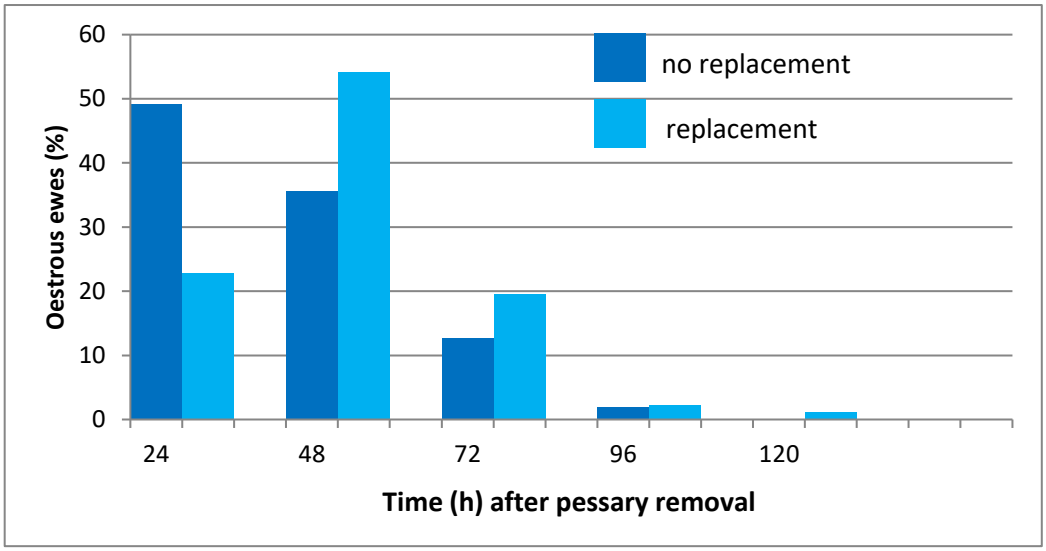
DEVELOPMENT OF IMPROVED TREATMENT PROTOCOLS

Major improvements in AI success rates depend on the development of more reliable protocols for synchrony of oestrus. Details are provided below of three promising strategies.

1. Replacement of pessaries on Day 9

Conventional pessary treatment provides inadequate progesterone during the last week of treatment. This affects the maturation of ovulatory follicles and contributes to a variable oestrus. Replacing pessaries on Day 9 results in a delayed but better synchrony (Figure 2). Importantly, replacement overcomes the effects of nutrition and season. In our experiments, pregnancy rates are improved by 10% provided the insemination time is optimal (in this case 48h after pessary removal). The cost of replacing CIDRs can be offset by re-using CIDRs after cleaning and sterilising – there is adequate progesterone in used pessaries but a surface film develops and this needs to be removed. Currently, pessary replacement is recommended for all AI programs.

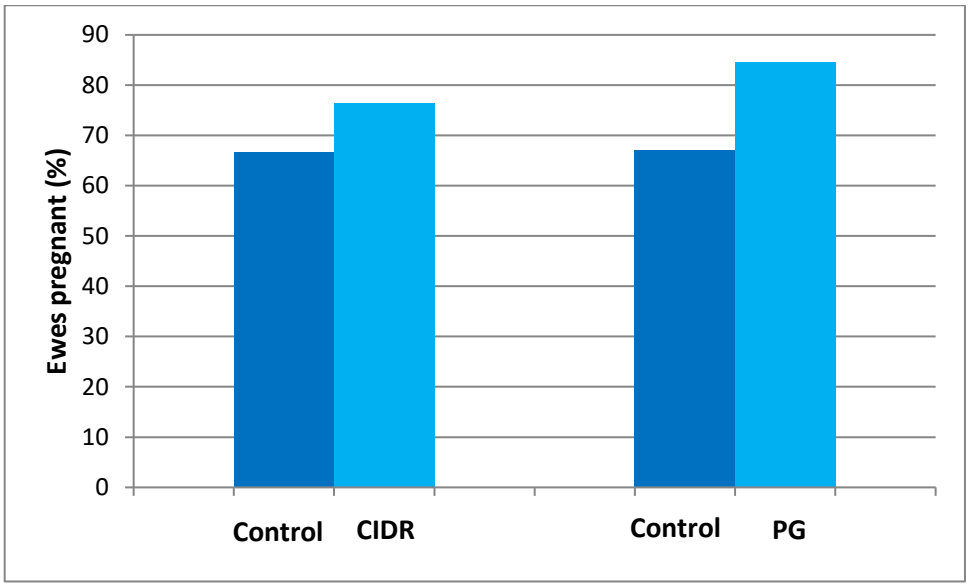
Figure 2. Effect of pessary replacement (on Day 9) on timing of oestrus.



2. Controlling the time of emergence of the ovulatory follicle

As indicated above, pregnancy is more likely if ovulatory follicles emerge during Days 7 – 9 of the pessary period. With conventional pessary treatment, there is no control over this timing but this can be achieved by pre-treating with either a CIDR or prostaglandin (PG). Pre-treatment with a CIDR involves inserting an initial CIDR 21 days before the second CIDR is inserted whilst pre-treatment with PG involves one injection 27 days before pessary insertion. With both pre-treatments, pregnancy rates are improved (Figure 3).

Figure 3. The effect of pre-treatment with either a CIDR or prostaglandin (PG) on pregnancy rates following AI with frozen-thawed semen.

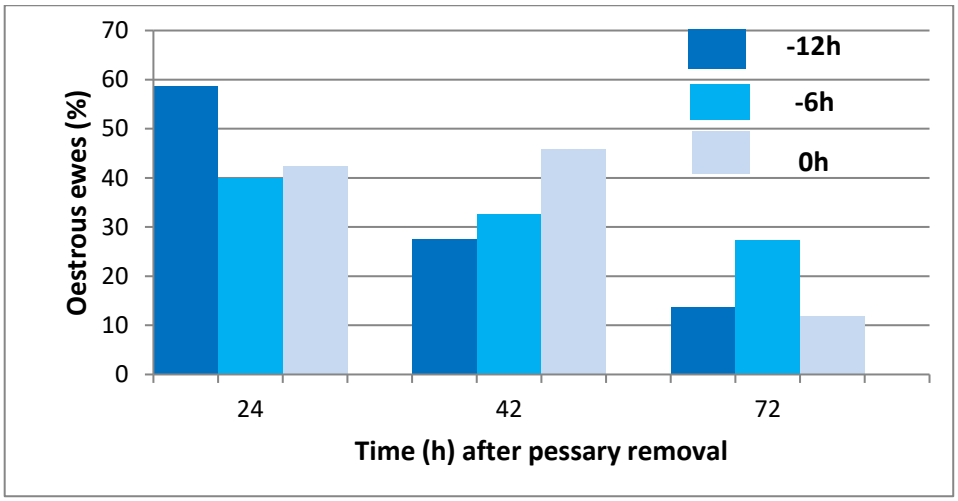


The potential lambing rate (fetuses/100 ewes inseminated) was not improved with the CIDR pre-treatment (due to a reduction in litter size) but was increased by 33.9% with the PG pre-treatment. This increase was due to improvements in both fertility and twinning rate and not to changes in the pattern of oestrus. Despite this encouraging result, the robustness of this treatment needs to be further evaluated before recommendations can be made on its usefulness.

3. Early treatment with PMSG

PMSG is usually given at pessary removal to stimulate follicle growth and to improve the synchrony of oestrus. However, treatment 12h beforehand results in a better but earlier synchrony (Figure 4). In this situation, an early insemination (from 42 – 43h after pessary removal) is essential. In our studies, this treatment resulted in a 17% increase in the number of fetuses per 100 ewes inseminated. Again, further research is required before an appropriate recommendation can be made.

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



MANAGEMENT PRACTICES TO IMPROVE AI OUTCOMES

There are several practises that can be employed.

1. Long and short-term nutrition

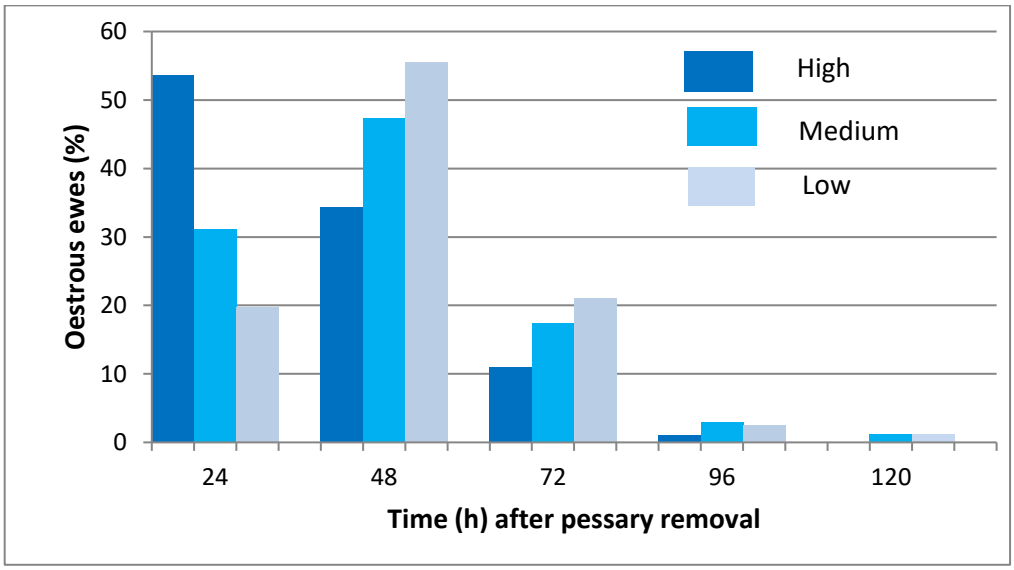
The entire period between the previous lambing and the cycle of AI is required for follicles to reach maturity. The prevailing nutrition during this period – **long-term nutrition** – affects the success of AI programs by influencing the pattern of oestrus. The effects of high nutrition (BCS 4.0^{+}) when compared with either medium (BCS 3.3^{+}) or low (BCS 2.7^{+}) nutrition are:

- Oestrus occurs earlier (Figure 5).
- 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 inseminated at 44h).
- Litter size is higher (1.50 versus 1.35 and 1.28 respectively).

On the other hand, high nutrition (1.5M) during the pessary period – **short-term nutrition** - increases pregnancy rate (79.0% versus 72.3% for 1.0M nutrition) but neither time of oestrus nor litter size are affected. An important point with short-term nutrition is that a 1.5M diet can moderate the effects of long-term nutrition (i.e. the differences in the timing of oestrus due to long-term nutrition are reduced). However, if pessaries are replaced on Day 9 (as is recommended), the

effects of nutrition are largely removed and a more reliable oestrus is produced. In this case, insemination needs to occur 48h after pessary removal irrespective of the level of nutrition.

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 and the cycle of AI.



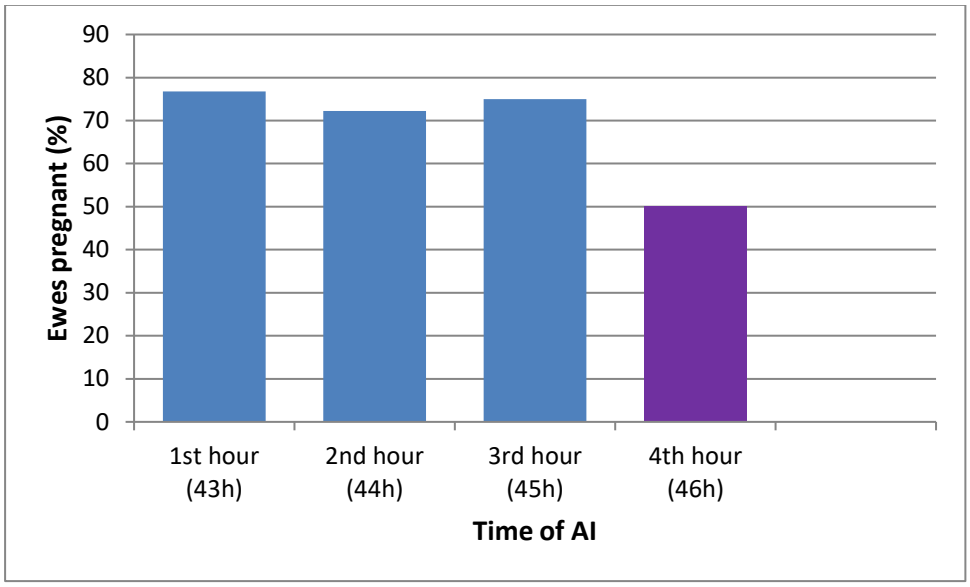
In addition, high nitrogen diets (e.g. lucerne, clover, lupin grain) should be avoided from two weeks before to two weeks after AI. These diets can produce excess ammonia that affects follicle/egg quality.

2. Managing ewes on the day of AI

Both anecdotal and research evidence indicate that pregnancy rates are higher when insemination occurs earlier in the day. This is exemplified in Figure 6 where pregnancy rates declined in ewes that were inseminated after the first three hours.

The reasons for this decline are not clear. However, until this finding is better understood, it is recommended that, in day-long programs, pessary removal be staggered (e.g. by 4 – 5h) to produce two comparable groups to be inseminated either in the morning or afternoon.

Figure 6. Effect of time of insemination on pregnancy rate (insemination with frozen-thawed semen commenced from approximately 42h after pessary removal).



3. Using teaser marks

Teasers fitted with harnesses/crayons should be run with the flock from the time of pessary removal. Crayon marks indicate when ewes are in oestrus and this information is useful:

- It indicates the normality of the synchrony.
- It enables ewes to be inseminated in approximate order of onset of oestrus. This helps to better match the times of insemination and ovulation.
- It identifies unmarked ewes which are generally less fertile than marked ewes. One practice is to inseminate these unmarked ewes with fresh semen should it be available.

In addition, teasers play an important role in stimulating follicle development, particularly in spring when a significant proportion of the flock is in anoestrus.

4. Maximising exposure to the ram effect

To maximise the ram effect, ewes can be placed in a shed with entire rams (physically separated) for up to 6h after pessary removal. This exposure potentially improves follicle maturation and aids in the onset of oestrus.

5. Culling dry ewes

Culling ewes known to have failed to lamb in the previous AI program can improve subsequent pregnancy rates.

TIME OF INSEMINATION

Incorrect timing of insemination is the major cause of poor pregnancy rates and this is a consequence of the variable patterns of oestrus. As mentioned above, the major factors responsible for the variable patterns of oestrus are:

- Nutrition - well-nourished flocks come into oestrus earlier than less-nourished flocks.
- Season – ewes in spring are later into oestrus than in autumn.
- Cyclicity - ewes that are not cycling (in spring) are later into oestrus than cycling ewes.

In addition, the following can also be involved:

- Age of ewe - maiden ewes come into oestrus about 6h earlier than mature ewes.
- Type of pessary – CIDRs induce oestrus about 6h earlier than sponges.
- Stress – little information is available but stress associated with yarding (e.g. dogs) after pessary removal has the potential to affect the onset oestrus.
- Inclement weather - consistently hot or cold/wet weather from about three days before pessary removal can affect the pattern of oestrus. For heat stress to be effective, it needs to be unrelenting with minimal relief at night and this is uncommon in most sheep areas of Australia.

Historically, with sponges, insemination usually commenced 48h after pessary removal – this timing preceded ovulation which usually commenced a few hours later. However, with CIDRs, ovulation occurs 6h earlier and this necessitates an earlier insemination time (i.e. from 42h). However, this change in insemination time generally hasn't happened and, as a consequence, pregnancy rates have probably suffered. Serendipitously, when pessaries are replaced on Day 9, the preferred insemination time is 48h.

TROUBLE SHOOTING

The pattern of oestrus indicates the well-being of the AI program and this is important in understanding why the program performed as it did (whether it be good or bad). The following factors help to characterise the pattern of oestrus:

- **No ewes in oestrus at 24h** – without pessary replacement, this is indicative of a delayed oestrus. The delay can be up to 12h and, if possible, insemination should be delayed.

However, with pessary replacement on Day 9, it is normal for few oestrous ewes to be observed at 24h.

- **Many ewes in oestrus at 24h** – this is generally not a problem but indicates insemination should occur early (e.g. 42h after pessary removal). Ewes can be in oestrus by 18h with up to 50% in oestrus by 24h. This situation does not occur when pessaries are replaced on Day 9.

- **Less than 50% of ewes in oestrus at insemination** – this indicates a poor synchrony and a likely poor pregnancy rate. One option is to not inseminate unmarked ewes or to use fresh semen if available. Poor nutrition or the grazing of oestrogenic pastures are likely causes.

- **Teaser lethargy** – this is possible if teasers are treated with testosterone for only two weeks and/or there are insufficient teasers. Longer treatment (e.g. four weeks) is preferred.

- **Some ewes in oestrus several days after AI** – these ewes are either very late coming into oestrus or have re-ovulated after the initial ovulation. They won't get pregnancy to AI but may conceive to backup rams.

- **Ewes showing oestrous behaviour but not attracting teasers** – this can occur in a small percentage of ewes (e.g. 5 - 7%). Some may eventually get marked.

- **Ewes producing mucus but not marked by teasers** – mucus can be observed up to 12h before the onset of oestrus. Ewes with globular opaque mucus are at this stage whilst ewes with clear mucus are generally in oestrus or approaching oestrus.

- **Flocks with a high incidence of uterine fluid** – this condition can only be observed with the laparoscope. It is more common in spring and can be (but not always) associated with the grazing of oestrogenic pastures. These ewes will not get pregnant to AI. The condition is temporary, lasting from a few weeks to several months.

THE PREFERRED PROTOCOL

Currently the preferred protocol involves the standard protocol (CIDR,PMSG) with pessaries replaced on Day 9. This results in a delayed but more synchronous oestrus with a need to commence insemination 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. PMSG. 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.

*Ropel can be given over a two-week period; treatment over four weeks is preferred because of improved teaser activity.

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