# The Phantom Cow Syndrome

**A study of cows that are not observed in oestrus within 24 days of an unsuccessful mating.**

*Final Report*

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# Executive Summary

- The Phantom Cow Syndrome is a major cause of sub-fertility in Australian dairy herds. A study in four commercial herds found that 22% of inseminated cows not pregnant to the first service did not return for a second service within 24 days. These cows have been defined as Phantom Cows.
- The fate of these Phantom Cows is that only 24% are likely to be pregnant to artificial insemination, 55% pregnant to the bull, and 21% remain not pregnant 21 weeks after the Mating Start Date (MSD).
- The Phantom Cow Syndrome is made up of many conditions, but may be categorised into nine categories. Of these, four categories make up 88% of the observed cases.
- The most common category (39% of Phantom Cows) comprises cows that have a long oestrous cycle after insemination, and are detected in oestrus 25 to 49 days after the first insemination.
- Another group of cows (21% of Phantom Cows) also appear to have a long oestrous cycle, and they are not observed in oestrus and have not been served a second time within 49 days of the first insemination. These first two categories may represent embryo mortality.
- A proportion of cows (19% of Phantom Cows) have a period of either anoestrus or an extended non-luteal phase. They are unable to show oestrus while they remain in this state.
- There was an observed pregnancy loss between cases observed pregnant at an early pregnancy diagnosis that subsequently were diagnosed not-pregnant at a 13-week examination (9% of Phantom Cows). This level of occurrence is similar to previous reports.
- Other categories of the Phantom Cow Syndrome (<5% incidence) include cows with no return within 49 days of insemination, cows that were likely to have been missed in oestrus, cows with an infection of the reproductive tract, a cystic ovary, or substantial adhesions.
- An important finding of this research is that few cases were attributed to failure of oestrus detection (7/380 cases). This indicates that failure to return to oestrus was real and not due to oestrous detection errors. This observation is specific to the study herds, and herds that are not competent in detecting oestrus would have an increased risk of the Phantom Cow Syndrome.
- Risk factors have been determined for Phantom Cows with the collaborative efforts of Dr John Morton and the InCalf Project. The strong factors identified with data from that study were the interval from calving to the mating start

date (MSD); the protein % of milk production; age; herd; carry over cows; retained foetal membranes and vaginal discharge; and the diagnosis and treatment of anoestrous cows.

- These risk factors have been modelled for the categories of Phantom Cows observed in the Maffra study. The importance of each risk factor may differ for each category but strong factors included diagnosis and treatment before the first insemination, the interval from calving to the mating start date (MSD), the protein % of milk production, and herd.
- Cows that were diagnosed as anoestrus before the MSD (and treated with a progesterone based protocol) had a greater risk of becoming Phantom Cows than cows that were inseminated without any prior treatment (27% vs 13%). Cows treated with a single injection of prostaglandin to induce the first oestrus had an intermediate incidence of Phantom Cows compared to cows that were not treated (17% vs 13%).
- The ability to resynchronise Phantom Cows was determined by progesterone concentrations between days 13 and 22 following insemination. Only 5% of cows treated with prostaglandin to synchronise oestrus could be resynchronised, whereas 47% of treated anoestrous cows could have been resynchronised.
- The two previous points contend that cows treated with a prostaglandin oestrous synchrony protocol are likely to have a greater incidence of Phantom cows than normally cycling cows, and these cows are less able to be resynchronised. These observations, if repeated, may need to be considered before recommending a prostaglandin synchrony protocol.
- The greater incidence of Phantom Cows in the anoestrous population has a number of implications. Firstly, the effect of these cows being anoestrus at the start of mating and the effect of treatment could not be differentiated in this study as it was not designed to test such differences. However, it could be expected that anoestrous cows have predisposing factors that mean they were more likely to become Phantom Cows. Secondly, the treatment used was not perfect, as a proportion of those cows either continued to be anoestrus, or lapsed into a state of anoestrus, after the first service. Thirdly, these cows were not resynchronised as is currently recommended. Resynchrony could be the most effective for this group, as it is possible to induce 47% of the Phantom Cows to express a second oestrus. This could increase the second round submission rate from 51% to 74%.
- The comparison of reproductive performance in the four herds with InCalf parameters showed that only one of the herds had typical results. Each of the four herds had excellent 3-week and 4-week submission rates. The factors limiting 6-week in-calf rates were below average first insemination conception rates and low second round submission rates.

- The research also evaluated the use of heat mount detectors to improve the second round submission rate. There was no benefit from the additional use of these devices compared to regularly observing tail paint and oestrous behaviour in the paddock.
- The changes in the herd profile of body condition scores are presented for each of the four herds. They each had a different profile that reflected feeding management decisions.

## Recommendations

- This research project has not identified a single major cause of the Phantom Cow Syndrome, nor a single preventative measure or treatment. Rather it has identified cows that are at greatest risk of becoming Phantom Cows and has categorised the various symptoms.
- The single most important preventative measure is to calve cows down to allow them sufficient time to recover before the next mating season. This minimises the risk of anoestrus and of becoming a Phantom Cow, and has been shown by the InCalf Project to increase the conception rate to first service and 6-week in-calf rates.
- All cows diagnosed as anoestrus should be treated before the MSD and should be resynchronised as is currently recommended. Anoestrous cows would still be likely to have a greater incidence of Phantom Cows, and further work is required to develop appropriate preventative strategies and improved treatments.
- The analyses of risk factors have identified the protein composition of milk as a strong factor of the incidence of both Phantom Cows and cows that are anoestrus before the MSD. This observation complements findings of the InCalf project that protein composition affects 6-week in calf rates and submission rates. More research is required to understand these relationships, particularly if protein composition relates the physiological state of the cow.
- It is noteworthy that oestrous detection errors as well as gross pathology of the reproductive tract (infections etc) are of minor significance compared to the long oestrous cycles and possible embryo mortality. Understanding the causes of long oestrous cycles and embryo mortality may increase the number of cows pregnant to the first service, as well as increase the number of non-pregnant cows are served again within 24 days.

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# Background

For some time there has been anecdotal evidence that a proportion of the herd are assumed to be in calf to AI but are later found to be not pregnant. The InCalf Project was the first to highlight this fact by measuring the "Second Round Submission Rate". This is the proportion of cows that were inseminated in the second 3-weeks of the mating season that had not conceived in the first 3-weeks. The typical InCalf herd only had a 69% second round submission rate.

This begs the question; what has happened to the other 31%? Are herd managers missing many oestrus events (heats)? Is there something wrong with these cows? The scale of this problem has not been acknowledged in any other country; but then again a study as powerful as the InCalf Project has not been completed overseas. This development is not likely to be confined to Australian conditions; it has not been quantified and considered as seriously in other countries.

The lack of knowledge of these "Phantom Cows" prompted a research project at the University of Melbourne that was funded by the Dairy Herd Improvement Fund. The data collected from this project has been used to complete a number of analyses. The detailed methodologies and the results are presented as manuscripts in the Appendices. This final report builds on these manuscripts by providing more farmer-oriented results and interpretations, and also provides extra analyses that have built on the results presented in the manuscripts.

## *A Phantom Cow field study*

A large field study of herds in the Maffra district profiled cows from when they calved until a pregnancy diagnosis in early 2001.

Cows were body condition scored around the time of calving, before the start of mating, and one month into mating. Abnormal events around the time of calving were recorded. Cows were tail painted from one month before the mating start date (MSD) so that herd owners could detect cows that had been in oestrus (on heat) before the start of mating. The herd's veterinarian examined those cows not detected in oestrus. In most cases they were diagnosed as anoestrus and treated with a standard anoestrus-CIDR protocol.

Heats and services for every cow in each herd were recorded. Those cows inseminated in the first 3 weeks (about 90% of the herd) were of particular interest. They had milk samples collected twice weekly from 14 days after insemination. Milk sampling continued either until the cow was seen in oestrus again, was confirmed pregnant, or stopped after seven weeks post-insemination if a cow was not pregnant and had not been seen in oestrus. Milk samples were assayed for progesterone to profile the length of luteal periods after insemination, and also to identify cows with prolonged periods without luteal activity.

A representative group of cows was selected for early pregnancy diagnosis. It was possible to observe a pregnancy by ultrasonography from 28 days after the first insemination. Those cows which were not observed as pregnant were checked again the following week. Only after both diagnoses were cows considered to be not pregnant to the first insemination.

The pregnant status of cows was again checked (this time for the whole herd) about 13 weeks after the MSD. Pregnancy losses were measured between the early diagnosis and the 13-week diagnosis.

## *Collaboration with the InCalf Project*

The InCalf project first highlighted the problem of cows not being detected for a second insemination. This rare database has provided the opportunity to further analyse the reproductive records of over 110 herds to determine risk factors that improve the chances of predicting which cows in the herd will be Phantom Cows.

Cows in the InCalf database were identified as either pregnant to the first service, returning to service within 24 days of first insemination, or as non-pregnant, non-return (NRNP) cows. Two models were developed. The first compared the NRNP cows with those that conceived to the first service, and the second compared the NRNP cows that returned within 24 days for a second service. The results of this project have been presented in Manuscript form in Appendix 3.

## *Continuation of the research program*

The results in this report demonstrate the significant impact of these Phantom cows on reducing the reproductive performance of dairy herds. However, there are many different conditions that lead to cows being classified as Phantom Cows, and there is not a single preventative measure or treatment to reduce the impact of this condition.

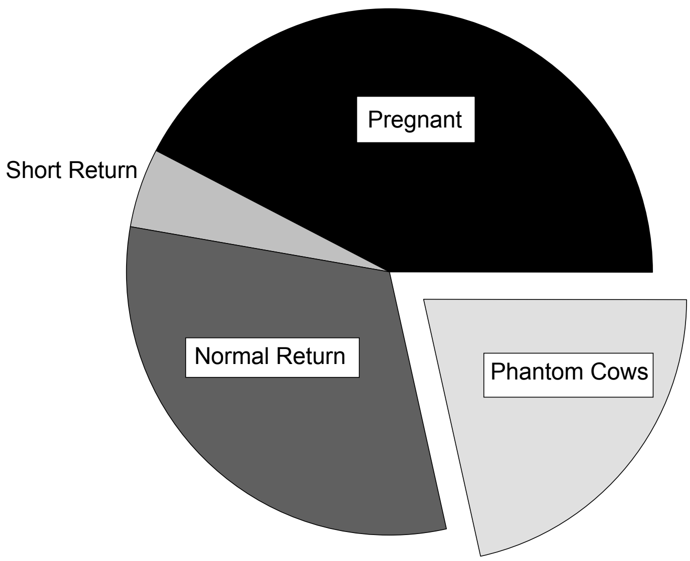
# Research Outcomes

## *Incidence of Phantom Cows*

Measuring the incidence of Phantom Cows depends on the post-insemination interval to the diagnosis of pregnancy, and when you consider that a cow not pregnant to the first service should have been observed in oestrus for a second service.

In this study, cows were considered pregnant if they were palpated as pregnant at 13 weeks. This measure therefore includes cows that were observed as pregnant at an earlier diagnosis but had since lost their pregnancy. The normal oestrous cycle of a cow is of 18 to 24 days duration, with an average of 21 days. It could be presumed that cows not conceiving to the first insemination should have returned for a second oestrus within 25 days.

Cows have been categorised as pregnant to the first service (Pregnant), returned to service 2 to 17 days later (Short Return), returned to service 18 to 24 days later (Normal Return), or were not pregnant and did not return within 24 days (Phantom Cows).




| | n | |
|---|---|---|
| Pregnant | 506 | 42% |
| Short Return | 58 | 5% |
| Normal Return | 373 | 31% |
| Phantom Cows | 257 | 22% |

## *There are many types of Phantom Cows!*

The 22% of cows that have been categorised as Phantom cows comprises cows that were diagnosed with many different conditions.

The figures presented in this report differ slightly from those in the preliminary manuscript (Appendix 1) because these data benefit from diagnoses made from the milk samples. A profile was built over time; first there was data on when (and if) cows returned to oestrus; then early pregnancy data; and finally, milk samples. Some data was contradictory. For example, some cows were presumed to be anoestrus at the early pregnancy diagnosis, but in fact were in that part of their oestrous cycle where they were about to show oestrus, when they can be confused with an anoestrous state. The milk profiles gave the most accurate indication of what happened to these cows, and this was used as the primary source of information for these categories. The observations at the early pregnancy diagnosis, and the herd owner's breeding records were used to complete the definitions.

This breakdown of the various conditions is by no means comprehensive but it is a summary of the different types of cows. For example, there were clearly six different types of cow categorised as anoestrus/prolonged non-luteal period. Each of these types would not consistently respond to the same treatment. The major point from this exercise was to demonstrate the large variation in conditions attributed to form a "Phantom Cow Syndrome".

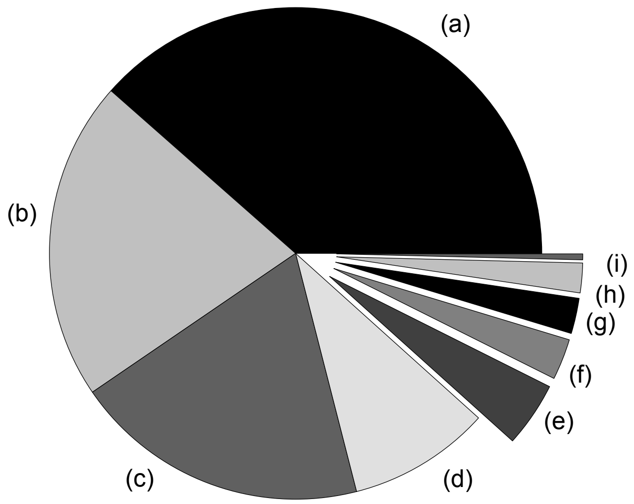
While there was a large degree of variation, it is also of interest that 88% of the Phantom cows could broadly be classified into four categories. There were:

a) cows that returned to oestrus between 25 and 49 days after insemination and were detected in oestrus (Long return – detected);
b) cows that appeared from milk samples to ovulate and/or show oestrus between 25 and 49 days after insemination but were not detected in oestrus (Long return – not detected);

c) cows with an abnormal hormone profile where there was an extended period of non-luteal progesterone concentrations after the first insemination indicating either an anoestrus state or a prolonged non-luteal period (Anoestrus/ Prolonged non-luteal period); and,

d) cows which were observed as pregnant at the early pregnancy diagnosis but were found at the 13-week examination to be not pregnant (Lost pregnancy)

Other categories included: cows with no evidence of a return to oestrus within 49 days of the first insemination (No return within 49 days); cows appearing to have been missed in oestrus around three weeks after the first service (Missed heat); cows with an infection in the reproductive tract (Pyometra); cows with a cystic ovary (Cyst); and cows with substantial uterine adhesions (Adhesions).




| | | n | |
|---|---|---|---|
| (a) | Long return - detected | 99 | 39% |
| (b) | Long return – not detected | 54 | 21% |
| (c) | Anoestrus/ Prolonged non-luteal period | 50 | 19% |
| (d) | Lost pregnancy | 24 | 9% |
| (e) | No return within 49 days | 11 | 4.3% |
| (f) | Missed heat | 7 | 2.7% |
| (g) | Pyometra | 6 | 2.3% |
| (h) | Cyst | 5 | 1.9% |
| (i) | Adhesions | 1 | 0.4% |

While this profile of the Phantom Cow Syndrome does not indicate that a single preventative measure or treatment would be effective, there are important observations on the nature of these categories.

Very few of the phantom cows were genuinely missed in oestrus around three weeks after the first insemination . Overall 373/380 (98%) of cows that returned to oestrus at this time were detected. This means that the herd owners from this study were competent in finding cows returning to oestrus. These herd owners do not need to consider more elaborate systems to detect cows in oestrus (see the later section on using Heat-mount detectors).

While the incidence of cows that were anoestrus or had prolonged non-luteal periods was a major category, it was mostly present in the group of cows that were anoestrus before the start of mating that received a CIDR treatment. It is important to note that some cows that were not diagnosed as anoestrus were also included in this category. This may be due to false heat detection in the pre-mating/ mating period when the cow was in an anoestrous state. Alternatively, some cows may revert to an anoestrus state after insemination. In either case, these cows would not be observed in a timely manner and would slip through the artificial breeding period without the opportunity for veterinary intervention such as resynchronisation of returns to service.

The cows that were anoestrus before mating were tracked through the mating period. These cows would normally be included in a re-synchrony program if current recommendations were followed. Resynchrony was not used in this study so as to observe cows and how they would spontaneously return to oestrus or remain anoestrus. The accurate identification of anoestrous cows (non-cycling or NVO) before the start of mating and appropriate treatment would reduce the impact of these cows on a herd's reproductive performance. Not only would they have an induced oestrus and ovulation after their period of anoestrus, they would also have a second chance at the time of resynchrony if the first treatment had not worked.

The number of cows that lost a pregnancy between an early diagnosis and the 13-week examination (n=24) was typical of the level of embryo mortality reported in other studies over the past 20 years. This indicates that "late" embryo mortality has not increased by comparison with previous studies. This does not preclude embryo mortality as a significant contribution to the problem; it only suggests that most cases of embryo mortality occur before 28 days.

The large proportion of cows returning between 25 and 49 days after insemination may be due to early embryo mortality. Such oestrous cycle lengths are abnormal. The extended luteal phases of these cycles demonstrated that it was the failure of luteolysis at the normal time that was a key factor. Failure to undergo luteolysis most commonly occurs when there has been maternal recognition of a pregnancy. Long oestrous cycles may be caused by embryo mortality after the dam has recognised the pregnancy and prevented luteolysis. It then takes an extended period of time for the dam to acknowledge the mortality and return to oestrus.

The failure of luteolysis at the normal time is an important area for future research and may provide real benefits in minimising the impact of this syndrome.

## *Fate of Phantom Cows*

The fate of cows in the Maffra field experiment cannot yet be determined. A final pregnancy diagnosis is required to determine which cows were pregnant to the bull and which are not pregnant.

The fate of Phantom Cows (NRNP cows) in the InCalf database can be compared with those cows that did not get pregnant to the first service but did return for a second insemination within 24 days. There is a dramatic difference in all the categories for the Phantom Cows, with fewer cows pregnant to AI, over half the cows pregnant to the bull, and an empty rate of 21%.

The fate of NRNP cows and those that were not pregnant to the first insemination but returned for a second oestrus within 24 days.

| | NRNP Cows | Cows that were not pregnant to the 1st insemination but returned to oestrus |
|---|---|---|
| Cows pregnant to AI | 24% | 65% |
| Cows pregnant to the bull | 55% | 26% |
| Cows not pregnant | 21% | 9% |

## *Risk factors for Phantom Cows*

There are two main sources of knowledge of risk factors for the Phantom Cow Syndrome. The first is collaborative work with the InCalf Project, where the study herds were examined in a powerful analyses. These risk factors were then used to analyse the major categories of Phantom Cows seen in the Maffra field experiment.

### Collaboration with the InCalf Project

A comprehensive report of risk factors for Phantom Cows is detailed in Appendix 3. This report is a joint project with Dr John Morton and the InCalf project. The manuscript is written in a style suitable for analyses by epidemiologists, and is not necessarily farmer-friendly. Instead we have attempted to report the results in a manner than can be used to interpret the importance of each risk factor.

Below are the risk factors that have the largest contribution towards Phantom Cows (NRNP cows). The final model included the effects of herd, the interval from calving to MSD, the protein% of milk, age, retained foetal membranes, vaginal discharge, and carrying cows over.

Each of these tables describes the relative incidence of Phantom Cows. For example, the interval from calving to MSD is considered after all of the other factors (age, herd etc) have been accounted for. This gives a better estimate of the true effects of this interval.

For each interval, such as at 9-12 weeks, there is an estimate of the Phantom cow incidence (16%) as well a 95% confidence interval (95% CI; 13% to 19%), and a relative risk (1.16). This means that we expect this group to have a 16% Phantom Cow incidence, and we are 95% confident that the true incidence is between 13 and 19%. In other words, cows in this group are 16% (i.e. 1.16) more likely to be phantom cows than cows calved more than 12 weeks.

## Calving to Mating Start Date Interval

| | No. cows | Relative NRNP incidence | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **More than 12 weeks** | 1150 | **14%** | | | Referent group |
| **9 - 12 weeks** | 6435 | **16%** | 13% | 19% | 1.16 |
| **6 - 9 WEEKS** | 4044 | **22%** | 18% | 26% | 1.56 |
| **3 - 6 weeks** | 2096 | **28%** | 23% | 33% | 2.01 |
| **3 weeks or less (incl. cows calved after MSD)** | 699 | **40%** | 33% | 47% | 2.89 |
| **Total** | 14424 | | | | |

For every three week decrease in the time from calving to the MSD there is a marked increase in the incidence of Phantom cows. This demonstrates that one of the main opportunities to prevent Phantom Cows is to ensure a compact calving period where all cows are given sufficient time from calving to the start of mating. It also highlights that late calvers may need extra attention during the breeding season and that their failure to return to oestrus after being bred does not mean that they are pregnant.

## Protein % of milk over the first 120 days of lactation

| | No. cows | Relative NRNP incidence | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **2.75 or less** | 259 | **22%** | | | Referent group |
| **2.75 - 3.00** | 2406 | **23%** | 17% | 30% | 1.06 |
| **3.00 - 3.25** | 5359 | **19%** | 14% | 25% | 0.88 |
| **3.25- 3.50** | 3898 | **18%** | 13% | 24% | 0.83 |
| **Higher than 3.50** | 2350 | **17%** | 12% | 23% | 0.75 |
| **Unknown** | 152 | | | | |
| **Total** | 14424 | | | | |

This is a remarkable trend because it has not been reported before as a strong factor and has resulted in much debate as to the significance of this relationship. At first glance it may be presumed that it might be associated with nutritional causes in early lactation. But when you consider that this relationship is measured **within** each herd and not across herds, and also within age, interval from calving to MSD etc, then this nutritional theory is less readily accepted. Further work by Dr Morton has shown that this relationship also holds for milk protein % over the whole lactation, not just 120 days. It now seems more likely that this might be a marker of genetic differences between cows that predispose them towards sub-fertility. This novel finding, and its consistency with the InCalf findings that it influences 6-week in-calf rates and other measures, deserves more research to determine its importance.

## Age

| | No. cows | Relative NRNP incidence | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **2 years** | 3020 | **18%** | | | Referent group |
| **3 years** | 2601 | **16%** | 14% | 18% | 0.90 |
| **4 years** | 2265 | **15%** | 13% | 17% | 0.84 |
| **5 - 7 years** | 4108 | **16%** | 14% | 18% | 0.87 |
| **Over 7 years** | 2008 | **20%** | 18% | 23% | 1.13 |
| **Unknown** | 422 | | | | |
| **Total** | 14424 | | | | |

Phantom cows are most likely to occur in older cows, followed by heifers, and are least likely to occur in middle aged cows. An explanation of this trend is given in manuscript in Appendix 3.

## Cows carried over

| | No. cows | Relative NRNP incidence | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **No** | 13050 | **18%** | | | Referent group |
| **At least 2 years before study** | 332 | **16%** | 12% | 21% | 0.92 |
| **Year before study** | 402 | **25%** | 20% | 31% | 1.44 |
| **Unknown** | 640 | | | | |
| **Total** | 14424 | | | | |

Of interest is the difference between cows carried over in past years, and those carried over the last season as compared to cows that had not been carried over. Those carried over the last season were more likely to become Phantom Cows.

Retained Foetal Membranes

| | No. cows | Relative NRNP incidence | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **No** | 13828 | **17%** | | | Referent group |
| **Yes** | 596 | **34%** | 29% | 39% | 1.94 |
| **Total** | 14424 | | | | |

Vaginal discharge

| | No. cows | Relative NRNP incidence | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **No** | 14354 | **18%** | | | Referent group |
| **2 weeks or less after calving** | 70 | **30%** | 19% | 44% | 1.72 |
| **Total** | 14424 | | | | |

These factors have been shown to be important because we have has access to such a large database. As can be seen above there were only 70 cases of vaginal discharge and 596 cases of retained foetal membranes out of 14,424 cows. The Maffra field experiment had too few numbers of cows to be able to make such comparisons.

## What risk factors are important in determining categories of Phantom Cows?

The Maffra field experiment is of a scale where common risk factors from the InCalf Project can be evaluated as well as body condition scores (which were not available for analysis in the InCalf Project). Risk factors can be derived for a number of groups of cows:

- Cows that are anoestrus before the start of mating
- Cows that are pregnant to the first service
- Cows that return for a second service within 24 days
- Phantom Cows
- Cows which lost a pregnancy between early pregnancy diagnosis and a 13-week examination
- Cows which had a period of anoestrus or a prolonged non-luteal period after the first insemination

- Cows which had a long oestrous cycle after the first insemination

This report has converted epidemiologic data into reports suitable for interpreting the importance of risk factors. The epidemiologic tables are presented in Appendix 4.

Separate models had to be developed because we could not consider the BCS at calving, and the BCS at the pre-mating visit at the same time. Two separate analyses are presented where there was a difference between calving BCS and pre-mating BCS.

## Risk factors associated with cows being anoestrus before the start of mating – a) A model including condition score at calving.

Separate models had to be developed because we could not consider the BCS at calving, and the BCS at the pre-mating visit at the same time. This model included herd, age, calving interval, protein % and BCS at calving.

Herds 1 and 2 had a similar level of anoestrus in their herds with fewer cases in Herds 3 and 4. Heifers were most at risk of being anoestrus, and there was a very strong effect of the interval from calving to MSD and protein % of milk production. Cows calved with a BCS less than 4.5 had the greatest chance of being anoestrus.

Herd

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **Herd 1** | 198 | **37%** | | | Referent group |
| **Herd 2** | 398 | **37%** | 28% | 48% | 1.00 |
| **Herd 3** | 395 | **29%** | 21% | 39% | 0.80 |
| **Herd 4** | 376 | **20%** | 14% | 28% | 0.53 |
| **Unknown** | 0 | | | | |
| **Total** | 1367 | | | | |

Age

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **2 years** | 263 | **35%** | | | Referent group |
| **3 years** | 222 | **31%** | 23% | 42% | 0.90 |
| **4 - 6 years** | 501 | **24%** | 18% | 32% | 0.69 |
| **Over 6 years** | 381 | **28%** | 21% | 36% | 0.79 |
| **Unknown** | 0 | | | | |
| **Total** | 1367 | | | | |

Interval from calving to the MSD

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4 to 6 weeks** | 131 | **70%** | | | Referent group |
| **7 to 9 weeks** | 405 | **38%** | 27% | 51% | 0.54 |
| **10 to 12 weeks** | 655 | **18%** | 12% | 27% | 0.26 |
| **13+ weeks** | 166 | **11%** | 6% | 19% | 0.16 |
| **Unknown** | 10 | | | | |
| **Total** | 1367 | | | | |

Milk Protein % in the first 120 days of lactation

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **2.75 or less** | 19 | **74%** | | | Referent group |
| **2.75 to 3** | 245 | **49%** | 16% | 83% | 0.66 |
| **3 to 3.25** | 620 | **39%** | 11% | 76% | 0.80 |
| **3.25 to 3.5** | 363 | **29%** | 7% | 67% | 0.39 |
| **3.5 and more** | 114 | **22%** | 5% | 61% | 0.30 |
| **Unknown** | 6 | | | | |
| **Total** | 1367 | | | | |

Body Condition Score at calving

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4.5 and less** | 321 | **42%** | | | Referent group |
| **4.75 to 5.25** | 584 | **39%** | 31% | 47% | 0.92 |
| **5.5 and greater** | 387 | **27%** | 20% | 37% | 0.65 |
| **Unknown** | 75 | | | | |
| **Total** | 1367 | | | | |

## Risk factors associated with cows being anoestrus before the start of mating – b) A model including condition score at a pre-mating visit.

The inclusion of BCS at the pre-mating visit changed the model. The effect of age was not a factor in this model whereas it was a factor in the BCS at calving model. This means that the BCS at calving is not adequately explained without also considering the age of the cow, whereas the trend of more anoestrus cows with lower BCS before mating does not need to also consider the age of the cow.

The other relationships were the same as the first model, that is that there were differences between Herds 1&2 and 3&4, and for the interval from calving to the MSD, and for the protein % of milk production.

Body Condition Score at a pre-mating visit

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4.5 and less** | 321 | **42%** | | | Referent group |
| **4.75 to 5.25** | 584 | **37%** | 30% | 44% | 0.87 |
| **5.5 and greater** | 387 | **25%** | 18% | 34% | 0.60 |
| **Unknown** | 75 | | | | |
| **Total** | 1367 | | | | |

Herd

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **Herd 1** | 198 | **37%** | | | Referent group |
| **Herd 2** | 398 | **38%** | 29% | 48% | 1.04 |
| **Herd 3** | 395 | **26%** | 19% | 35% | 0.71 |
| **Herd 4** | 376 | **22%** | 16% | 30% | 0.60 |
| **Unknown** | 0 | | | | |
| **Total** | 1367 | | | | |

Interval from calving to the MSD

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4 to 6 weeks** | 131 | **70%** | | | Referent group |
| **7 to 9 weeks** | 405 | **43%** | 32% | 54% | 0.61 |
| **10 to 12 weeks** | 655 | **22%** | 15% | 31% | 0.31 |
| **13+ weeks** | 166 | **14%** | 8% | 23% | 0.20 |
| **Unknown** | 10 | | | | |
| **Total** | 1367 | | | | |

Protein % of milk production

| | No. cows | Anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **2.75 or less** | 19 | **74%** | | | Referent group |
| **2.75 to 3** | 245 | **45%** | 14% | 80% | 0.60 |
| **3 to 3.25** | 620 | **37%** | 11% | 74% | 0.83 |
| **3.25 to 3.5** | 363 | **28%** | 7% | 66% | 0.38 |
| **3.5 and more** | 114 | **23%** | 5% | 61% | 0.30 |
| **Unknown** | 6 | | | | |
| **Total** | 1367 | | | | |

## Risk factors associated with pregnancy to the first service.

There was a difference between herds in the pregnancy rate to first service. This was measured at the 13-week examination, so does not include those cows that lost the pregnancy between the early diagnosis and the 13-week examination (these cows are part of the Phantom Cow Syndrome). Herd 3 had a better pregnancy rate than the other herds. This is of interest as the same AI technician worked with Herd 3 and Herd 1.

This trial has produced similar results to the InCalf study, with better pregnancy rates observed in cows with longer intervals from calving to MSD, and with higher protein composition of milk. This confirms the importance of calving cows as early as possible to give the more time to recover and prepare for the next pregnancy. It also supports the InCalf results that milk protein is a new indicator of reproductive performance, but that is poorly understood and in need of more research to understand this relationship.

### Herd

| | No. cows | Pregnant to 1st serve | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **Herd 1** | 171 | **40%** | | | Referent group |
| **Herd 2** | 379 | **46%** | 36% | 56% | 1.15 |
| **Herd 3** | 361 | **50%** | 40% | 59% | 1.25 |
| **Herd 4** | 321 | **39%** | 31% | 49% | 0.99 |
| **Unknown** | 135 | | | | |
| **Total** | 1367 | | | | |

### Interval from calving to the MSD

| | No. cows | Pregnant to 1st serve | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4 to 6 weeks** | 119 | **29%** | | | Referent group |
| **7 to 9 weeks** | 356 | **39%** | 29% | 51% | 1.33 |
| **10 to 12 weeks** | 598 | **46%** | 35% | 57% | 1.57 |
| **13+ weeks** | 155 | **48%** | 35% | 61% | 1.64 |
| **Unknown** | 139 | | | | |
| **Total** | 1367 | | | | |

Protein % of milk production

| | No. cows | Pregnant to 1st serve | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **2.75 or less** | 15 | **14%** | | | Referent group |
| **2.75 to 3** | 218 | **28%** | 8% | 65% | 2.02 |
| **3 to 3.25** | 557 | **33%** | 10% | 69% | 1.17 |
| **3.25 to 3.5** | 330 | **41%** | 13% | 76% | 2.93 |
| **3.5 and more** | 107 | **44%** | 14% | 79% | 3.13 |
| **Unknown** | 140 | | | | |
| **Total** | 1367 | | | | |

### Risk factors associated with cows not pregnant to the first service but returning to oestrus within 24 days.

It is of note that the only factor that could be related to cows returning to serve after failing to conceive is a herd difference. And this difference is not strong, with all the herds having a distribution that overlapped (this can be seen by all the confidence intervals overlapping and also including the first reference value).

So there is no strong factor that is associated with detecting more non-pregnant cows on heat within three weeks of the first insemination.

Herd

| | No. cows | Return to serve | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **Herd 1** | 171 | **36%** | | | Referent group |
| **Herd 2** | 379 | **38%** | 30% | 48% | 1.07 |
| **Herd 3** | 361 | **28%** | 21% | 36% | 0.78 |
| **Herd 4** | 321 | **43%** | 34% | 53% | 1.20 |
| **Unknown** | 135 | | | | |
| **Total** | 1367 | | | | |

## Risk factors associated with Phantom Cows.

A strong factor influencing the number of Phantom Cows was their diagnosis and treatment before the first insemination. Cows that were not detected in oestrus (no visible oestrus; NVO) had a greater incidence of Phantom Cows whether they did not have a palpable CL and were treated with a CIDR device (NVO CIDR), or they did have a palpable CL and were not treated (NVO CL). The NVO CL group were only diagnosed in Herds 3 and 4.

It is likely that the PG treated group has a greater incidence of Phantom Cows than cows that were not treated before the first insemination. This is an interesting observation when viewed in context of the observations in the Resynchrony chapter (p 42).

The fact that herd was not in this final model is noteworthy. This means that even though there were herd differences in pregnancy to first service and no difference in the population returning for a second service, there was no effect on the incidence of Phantom Cows. This implies that herds have a similar incidence of Phantom Cows regardless of conception rates.

Later sections of this analyses include herd as a factor in categories of Phantom Cows. It is only at this level (of breaking up Phantom Cows into categories) that we can conclude that there are differences between herds. Overall, all herds in this study had an approximate incidence of Phantom cows of 20%.

Diagnosis and Treatment before the first insemination

| | No. cows | Phantom cows | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **No Trt** | 363 | **13%** | | | Referent group |
| **PG** | 424 | **17%** | 12% | 23% | 1.27 |
| **NVO CIDR** | 407 | **27%** | 20% | 36% | 2.05 |
| **NVO CL** | 38 | **26%** | 13% | 44% | 1.93 |
| **Unknown** | 135 | | | | |
| **Total** | 1367 | | | | |

Interval from calving to the MSD

| | No. cows | Phantom cows | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4 to 6 weeks** | 119 | **33%** | | | Referent group |
| **7 to 9 weeks** | 356 | **30%** | 21% | 42% | 0.92 |
| **10 to 12 weeks** | 598 | **24%** | 16% | 34% | 0.72 |
| **13+ weeks** | 155 | **21%** | 12% | 34% | 0.63 |
| **Unknown** | 139 | | | | |
| **Total** | 1367 | | | | |

## Risk factors associated with a lost pregnancy between early pregnancy diagnosis and a 13-week examination.

Herd 1 had a greater loss of pregnancy then Herd 3, which is the opposite trend to that of Herd 3 having a greater pregnancy rate than Herd 1. This is in the context of the same AI technician used for both herds, but semen was sourced from different resellers and stored differently.

Body Condition Score is an interesting factor, with it being significant when measured at calving, but not when measured at a pre-mating visit. Cows with a high condition score at calving (≥5.5) were more likely to lose a pregnancy between the early diagnosis and the 13-week examination.

Herd

| | No. cows | Lost Pregnancy | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **Herd 1** | 171 | **5%** | | | Referent group |
| **Herd 2** | 379 | **3%** | 1% | 8% | 0.55 |
| **Herd 3** | 361 | **1%** | 0% | 3% | 0.22 |
| **Herd 4** | 321 | **2%** | 1% | 7% | 0.44 |
| **Unknown** | 135 | | | | |
| **Total** | 1367 | | | | |

Body Condition Score at calving

| | No. cows | Lost Pregnancy | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4.5 and less** | 295 | **1%** | | | Referent group |
| **4.75 to 5.25** | 524 | **2%** | 1% | 6% | 1.28 |
| **5.5 and greater** | 352 | **5%** | 1% | 15% | 3.52 |
| **Unknown** | 196 | | | | |
| **Total** | 1367 | | | | |

## Risk factors associated with a period of anoestrus or a prolonged non-luteal period after the first insemination.

Herd 4 was not involved in the milk sample program and so information was not available from this farm. Herd 3 had a greater incidence of cows that became anoestrus or had a prolonged non-luteal period, even though it had a lower incidence of anoestrus cows and a greater pregnancy rate to the first service.

Cows than were not observed in oestrus before the start of mating and treated with a CIDR device (NVO CIDR) had a greater incidence of anoestrus cows after the first insemination. This indicates that the treatment is not ideal and does not consistently induce regular oestrus cycles after the first insemination. It must be remembered that cows were not resynchronised in this study, and this resynchrony process will provide an opportunity to induce a second oestrus in those cows that did not conceive to the first insemination. This study shows how important it is to complete the recommended program that both synchronises **and** resynchronises NVO cows.

Some cows that were not diagnosed as NVO before the start of mating did have a period of anoestrus after the first insemination. Cows were more likely to have this anoestrus period if they had a short interval from calving to MSD and low protein composition of milk.

Herd

| | | No. cows | Post-insem anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|---|
| | **Herd 1** | 163 | **3%** | | | Referent group |
| | **Herd 2** | 361 | **2%** | 1% | 7% | 0.75 |
| | **Herd 3** | 343 | **10%** | 3% | 24% | 3.12 |
| | **Unknown** | 500 | | | | |
| **Total** | | 1367 | | | | |

## Diagnosis and Treatment before the first insemination

| | No. cows | Post-insem anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **No Trt** | 259 | **2%** | | | Referent group |
| **PG** | 354 | **0%** | 0% | 2% | 0.12 |
| **NVO CIDR** | 349 | **6%** | 2% | 15% | 3.11 |
| **Unknown** | 405 | | | | |
| **Total** | 1367 | | | | |

## Interval from calving to the MSD

| | No. cows | Post-insem anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4 to 6 weeks** | 113 | **12%** | | | Referent group |
| **7 to 9 weeks** | 298 | **10%** | 4% | 22% | 0.87 |
| **10 to 12 weeks** | 475 | **5%** | 2% | 13% | 0.44 |
| **13+ weeks** | 97 | **3%** | 0% | 20% | 0.24 |
| **Unknown** | 481 | | | | |
| **Total** | 1367 | | | | |

## Protein % of milk production

| | No. cows | Post-insem anoestrus | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **2.75 or less** | 14 | **29%** | | | Referent group |
| **2.75 to 3** | 186 | **15%** | 3% | 45% | 0.50 |
| **3 to 3.25** | 430 | **5%** | 1% | 22% | 0.36 |
| **3.25 to 3.5** | 264 | **3%** | 1% | 19% | 0.12 |
| **3.5 and more** | 93 | **3%** | 0% | 29% | 0.10 |
| **Unknown** | 380 | | | | |
| **Total** | 1367 | | | | |

## Risk factors associated with a long oestrous cycle after the first insemination – a) The oestrus was detected.

Herd 2 was less likely to have cows that had a long oestrous cycle after the first insemination that were detected in oestrus. There was again a relationship with interval from calving to MSD and protein composition of milk.

Cows with a long oestrus cycle had a corresponding long luteal phase, and it was the failure of luteolysis that caused the long oestrous cycle. Failure of luteolysis may be due to cows recognising a pregnancy, then suffering embryo mortality, but taking an extended period to acknowledge their non-pregnant state.

### Herd

| | No. cows | Long Return | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **Herd 1** | 163 | **8%** | | | Referent group |
| **Herd 2** | 361 | **3%** | 1% | 7% | 0.38 |
| **Herd 3** | 343 | **8%** | 4% | 16% | 1.05 |
| **Unknown** | 500 | | | | |
| **Total** | 1367 | | | | |

### Interval from calving to the MSD

| | No. cows | Long Return | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **4 to 6 weeks** | 99 | **6%** | | | Referent group |
| **7 to 9 weeks** | 252 | **5%** | 2% | 13% | 0.91 |
| **10 to 12 weeks** | 426 | **3%** | 1% | 8% | 0.51 |
| **13+ weeks** | 89 | **3%** | 1% | 10% | 0.46 |
| **Unknown** | 501 | | | | |
| **Total** | 1259 | | | | |

Protein % of milk production

| | No. cows | Long Return | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **2.75 or less** | 11 | **14%** | | | Referent group |
| **2.75 to 3** | 161 | **13%** | 2% | 57% | 0.92 |
| **3 to 3.25** | 382 | **6%** | 1% | 39% | 0.51 |
| **3.25 to 3.5** | 227 | **4%** | 0% | 32% | 0.31 |
| **3.5 and more** | 83 | **6%** | 1% | 41% | 0.40 |
| **Unknown** | 503 | | | | |
| **Total** | 1367 | | | | |

## Risk factors associated with a long oestrous cycle after the first insemination – b) The oestrus was not detected.

Herd was the only significant factor, and again it is a weak factor with no clear differences between herds.

The lack of other factors being included in the final model implies that there is not a strong factor that we can use to explain the incidence of cows that had a long oestrus cycle (and thus potentially embryo mortality) that are then not detected at the next oestrus.

Herd

| | No. cows | Returned without detection | 95% CI | | Relative Risk |
|---|---|---|---|---|---|
| **Herd 1** | 163 | **4%** | | | Referent group |
| **Herd 2** | 361 | **6%** | 3% | 14% | 1.71 |
| **Herd 3** | 343 | **3%** | 1% | 8% | 0.77 |
| **Unknown** | 500 | | | | |
| **Total** | 1367 | | | | |

## *Comparison of Maffra trial herds with InCalf parameters for typical herds*

The final pregnancy tests have not been completed for the Maffra field experiment, so the parameters presented are for the first seven weeks of mating.

| | Typical InCalf herd | Achievable target | Herd 1 | Herd 2 | Herd 3 | Herd 4 |
|---|---|---|---|---|---|---|
| 6-week in-calf rate | 63% | 75% | 57% | 58% | 64% | 52% |
| 7-week in-calf rate | - | - | 62% | 65% | 69% | 59% |
| 3-week submission rate | 77% | 87% | 87% | 93% | 92% | 87% |
| 4-week submission rate | - | - | 96% | 95% | 98% | 95% |
| 2nd round submission rate | 69% | 78% | 63% | 63% | 58% | 69% |
| First insemination conception rate | 49% | 54% | 39% | 39% | 49% | 36% |

Herd 3 has the best 6-week in-calf rate of the four herds. It is the only herd to be above the typical (or median) performance of the InCalf study herds. The other herds were at a large disadvantage with 6-week in-calf rates less than 60%.

The main reason for this difference is the first insemination conception rate, where Herd 3 had a typical result and the other herds had conception rates less than 40%. All herds had achieved an excellent 3-week submission rate, especially as those cows that had not been submitted at this time had already been examined by the veterinarian and were in the middle of a treatment for anoestrus. By 4-weeks over 95% of all cows in each herd were submitted.

It is of interest that the herd with the best results (Herd 3) also had a lower 2$^{nd}$ round submission rate. Thus, of the cows that were not pregnant to the first service, fewer cows were able to be bred to conceive to a second insemination. One reason for this difference was that Herd 3 had a greater incidence of cows that were anoestrus after the first insemination (page 32).

The use of 6-week in-calf rates is of lesser benefit than 7-week in-calf rates in these herds. This is because of the use of synchrony (both a prostaglandin or “why wait” protocol and a progesterone treatment of anoestrous cows protocol) induced many cows into oestrus in the 1st week of mating. Those cows that did not get pregnant to the first service should return in the 4th week (3 weeks later), and again in the 7th week. The other reason is that farmers often consider that those cows pregnant in the first seven weeks will be left to calve without treatment, while later pregnancies will be induced to calve.

As can be seen from the table, there is an increase from 6-week to 7-week in-calf rates that can be attributed to the original synchrony protocols. It is proposed that herds with pro-active management and widespread use of synchrony protocols might be better compared with the 7-week parameter.

## Return interval analysis

The low second round submission rate demonstrates the need to more closely interpret the time taken for non-pregnant cows to be presented for a second insemination.

A return interval (RI) analyses for those cows that did not conceive to the first insemination.

| | Herd 1 | Herd 2 | Herd 3 | Herd 4 |
|---|---|---|---|---|
| 2 to 17 day Return Interval | 12% | 6% | 4% | 15% |
| 18 to 24 day Return Interval | 51% | 57% | 54% | 54% |
| 25 to 38 day Return Interval | 21% | 12% | 16% | 6% |
| 39 to 45 day Return Interval | 1% | 7% | 4% | 2% |
| No second serve within 45 days | 15% | 18% | 22% | 23% |

The second round submission rate is the addition of the first two rows. It is noteworthy that only approx. 50% of non pregnant cows return at the expected 18 to 24 day interval.

The increased proportion of cows in the 2 to 17 day groups for Herd 1 and Herd 4 may represent inaccurate heat detection at the first or second insemination, whereas Herds 2 and 3 may be considered more typical. Herd 1 also had a greater incidence of cows returning at 25 to 38 day intervals.

The cows returning at 39 to 45 days have traditionally been categorised separately as they are presumed to represent cows missed at 18 to 24 days and returning another 3 weeks later (missed heats). This measure is presented in the table but it has been shown to be a false representation of missed heats. The milk samples collected demonstrate that the vast majority of these cows were not able to show oestrus at 18 to 24 days, and their first opportunity was in the 39 to 45 day interval.

It is of concern that approx. 20% of cows have not had a second chance for an insemination within 45 days of the first service. This group is then destined to be induced to calve if they do eventually get pregnant, and they are also likely to end up not pregnant (empty). In either case they are a significant cost to the enterprise.

## *Potential to resynchronise Phantom Cows*

In the last section it was demonstrated that only about half of the cows not pregnant to the first service were served for a second time 18 to 24 days after first service (as might be expected).

Phantom Cows are theoretically possible to resynchronise if the period of progesterone treatment overlaps a luteal phase, and the progesterone device is removed in a low progesterone environment where a CIDIROL injection can induce oestrus and an ovulation. Alternatively, a profile where there is consistently low progesterone (i.e. anoestrus) could respond to a standard resynchrony protocol.

Milk progesterone profiles can describe the suitability of such a resynchrony treatment. Twice-weekly samples have been categorised into high and low values. Low progesterone concentration was determined by the lower limit of 2 standard deviations from the mean of luteal concentrations on Days 13 to 16 (3ng/ml). Each cow has a profile based on progesterone concentration for Days 13-16, Days 17 to 19, and Days 20 to 22. The possible combinations of profiles that were observed are presented below.

Profiles based on high and low progesterone between Day 13 and Day 22 after insemination (n=150).

| Profile Type | n | Days 13 to 16 | Days 17 to 19 | Days 20 to 22 | Possible to Resynchronise |
|---|---|---|---|---|---|
| A | 93 | High | High | High | No |
| B | 25 | High | High | Low | Yes |
| C | 2 | High | Low | High | No |
| D | 7 | High | Low | Low | Yes |
| E | 3 | Low | High | High | No |
| F | 2 | Low | High | Low | Yes |
| G | 3 | Low | Low | High | Yes |
| H | 15 | Low | Low | Low | Yes |

Profile Type A represents cows with a prolonged luteal phase which would not be suitable for resynchrony. Profile types B and D represent the decrease in progesterone associated with the end of a luteal phase and would be suitable for resynchrony.

Profile H represents anoestrous cows which may respond to the resynchrony treatment.

The distribution of cows for each treatment and their potential to respond to resynchrony.

| Treatment | Possible to Resynchronise | | |
|---|---|---|---|
| | No | Yes | |
| No Treatment | 18 | 10 | (36%) |
| PG | 35 | 2 | (5%) |
| NVO CIDR | 45 | 40 | (47%) |
| **Overall** | **98** | **52** | **(35%)** |

It is of concern that only 5% of Phantom Cows from a PG treatment protocol have the potential for resynchrony. While the potential of NVO CIDR cows is 47%, in practice this is not achieved, presumably because some anoestrous cows do not respond to the synchrony protocol.

## Second round submission rate with and without resynchrony

We can estimate the maximum possible second round submission rate if all cows that are possible to resynchronise respond to treatment. From the table below, there were 688 cows not pregnant to the first serve, of which 431 (58 + 373) returned for a second service within 24 days. That left 257 cows diagnosed as Phantom Cows. The second round submission rate without resynchrony is 431/688 (63%).

Of the 257 Phantom Cows there were 35% that could theoretically be resynchronised. This leaves 167 cows that would not be submitted at the second round (a 76% submission rate with resynchrony).

This demonstrates that a suitable resynchrony program could improve submission rates by 13%. In practice there is not a treatment that is 100% effective, but progesterone based treatments should be considered.

Resynchrony is a crucial part of treating NVO cows. This table shows the potential to increase the second round submission rate from 51% to 74%. This confirms many previous studies by the Dairy Cattle Fertility group demonstrating the value of the resynchrony component of treating NVO cows before the start of mating.

| | *Overall* | NoTrt | PG | NVO CIDR |
|---|---|---|---|---|
| All cows not pregnant to first serve | *688* | 194 | 222 | 272 |
| Short return (2 to 17 days) | *58* | 21 | 20 | 17 |
| Normal Return (18 to 24 days) | *373* | 124 | 127 | 122 |
| Phantom Cows | *257* | 49 | 75 | 133 |
| Proportion of Phantom Cows that are able to be Resynchronised? | *35%* | 36% | 5% | 47% |
| Non-responders to resynchrony | *167* | 31 | 70 | 70 |
| Second Round SR **without** Resynchrony | 63% | 75% | 66% | 51% |
| Second Round SR **with** Resynchrony | *76%* | 84% | 68% | 74% |

## Effect of timing of Why-Wait treatment

A significant observation from the above table is the inability to resynchronise Phantom Cows that were treated with prostaglandin (PG) before the first insemination (5% chance). This observation is even more significant when combined with the increased chance of this group being categorised as Phantom Cows (see an earlier section).

Prostaglandin treatment does terminate the luteal phase and induce an oestrus earlier than would happen without treatment. The stage of the oestrous cycle at treatment might affect the fate of these cows. The following table has categorised PG treated cows by the interval from the last recorded pre-mating heat to the day of treatment (i.e. the day of the ensuing oestrous cycle). Cows that have an interval greater than 21 days were already overdue to have been in oestrus.

There was no apparent effect of the interval from the last pre-mating heat to PG treatment on the incidence of Phantom Cows. This indicates that the timing of PG could not be modified to reduce the impact of Phantom Cows.

Farmers that use prostaglandin to synchronise cows need to be aware that this treatment is increasing the number of Phantom Cows, and these cows have little chance of being resynchronised. These problems needs to be weighed up with the value of such a treatment to increase submission rates, and to promptly identify cows that have not been bred and need veterinary attention.

| Interval from last pre-mating heat to PG injection | % Phantoms | |
|---|---|---|
| 7 to 13 days | 17% | (35/211) |
| 14 to 20 days | 15% | (9/60) |
| over 21 days | 19% | (8/42) |

# Other Outcomes

## *Use of Heat-mount detectors to improve the submission of non-pregnant cows for a second service*

Question: Is there a benefit to use a Kamar® device as well as tailpaint to help detect cows returning to oestrus (heat)?

Answer: No!

The InCalf project identified farmers that used Kamar® devices to detect cows on heat at the start of mating found more cows in oestrus (they had a higher submission rate). This result was on the basis of a survey of all InCalf herds, and not measured specifically for each cow and what heat detection aid was used. It is of interest whether using Kamar® devices, as well as tail paint and paddock observation, will improve the herd owner's ability to find cows returning to oestrus for a second insemination.

### Trial design

Cows (n=153) were selected from two herds in the Maffra district in November, 2000. They were suspected to be more difficult to detect in oestrus (heat) about three weeks after they were first inseminated. The selection criteria were:

1. Cows with a body condition score of less than 4.5 at calving
2. Cows eight years old and older
3. Cows calved less than 43 days before the start of the mating season
4. Cows in the lowest quartile (25%) of milk protein production at the first herd test (as a % of yield)
5. Cows that were inseminated after an anoestrous treatment, but were originally found to have a small, inactive left ovary

They were randomly allocated to two groups, balanced for the five risk factors:

Group 1. Repainting the tailpaint two weeks after the first insemination so that it was fresh when the cow might return to oestrus (Tailpaint)

Group 2. Repainting the tailpaint as well as affixing a Kamar® device two weeks after first insemination (Tailpaint + Kamar)

Cows which were pregnant to the first service were not considered in the analysis. Those cows due to return to oestrus three weeks after the first insemination were observed both for signs of wear on the tailpaint or Kamar® device as well as observed in the paddock. Cows observed in oestrus were inseminated once a day by the same technician.

## Results

The number of cows bred 18 to 24 days after the first insemination is described in Table 1. There was no statistically significant difference between the Kamar + Tailpaint group and the Tailpaint group even though there was slightly higher numerical difference in the Tailpaint group. The fact that only about half of the non-pregnant cows were observed in oestrus three weeks later for a second insemination is of great concern.

The number of non-pregnant cows that were inseminated 18 to 24 days after first service (not significantly different).

| | Kamar + Tailpaint | Tailpaint |
|---|---|---|
| Cows inseminated | 28 | 47 |
| Cows not inseminated | 35 | 43 |
| Submission Rate | 44% | 52% |

For those cows that were bred at this time, the number that conceived to the second insemination is described in Table 2. There was no difference in the conception rates between the two groups. This is a crude indication that neither group had more cows that were falsely detected in oestrus.

The number of submitted cows that were pregnant to the second insemination (not significantly different).

| | Kamar + Tailpaint | Tailpaint |
|---|---|---|
| Cows pregnant | 18 | 29 |
| Cows not pregnant | 10 | 18 |
| Conception Rate | 64% | 62% |

The two herd owners did not find an advantage in using Kamar® devices as well as tailpaint. The added cost, and the extra work to apply the patches was not justified. However, other farmers may not have the same ability to detect worn tailpaint or cows in oestrus in the paddock, and might gain confidence in heat detecting with the help of Kamar® devices.

This study illustrates how farmers that are competent in using both tailpaint and observation of cows in the paddock do not get better results from using Kamar® devices. This study also emphasises that even with heat detection aids and paddock observation, only about half of the cows that were not pregnant to the first service were observed in oestrus three weeks later.

## *A case study of Body Condition Scores in four commercial herds in the Maffra district.*

Cows were condition scored within seven days of calving, one month before the MSD, and one month after the MSD. This has developed a herd profile of condition score changes through the first half of the season.

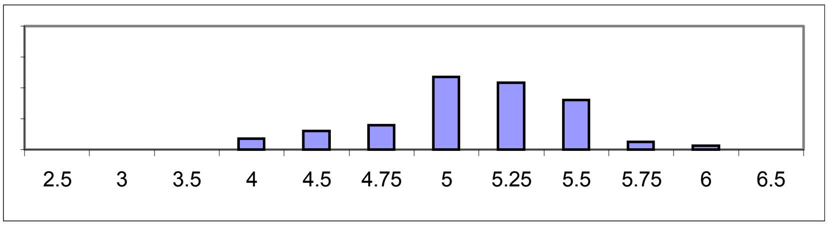
Each herd had differing management policies, labour units, and attitudes to feeding both dry cows and cows in early lactation. This has resulted in four different profiles of condition scores.

The following four pages describe each herd, with a distribution graph and key statistics of mean, and spread around the mean (standard deviation). The profiles have also been categorised into cows with a BCS of 4.5 or less, a BCS of 4.75 to 5.25, or a BCS of 5.5 or more.

Ideally cows would calve with a condition score of 5 or greater, with a slight decrease after calving and a rising plane through the mating period. In practice there are many different scenarios that cows experience from calving through to mating.

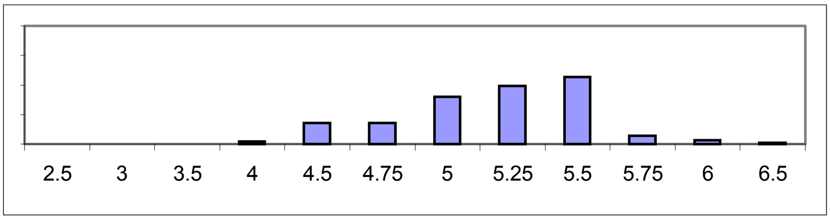
The profiles presented below do not describe what happens to individual cows, rather they represent the broad trends in the herd. The descriptions of each herd situation reflect the predominant trends in the herd and are not necessarily true for all cows in the herd.

Herd 1



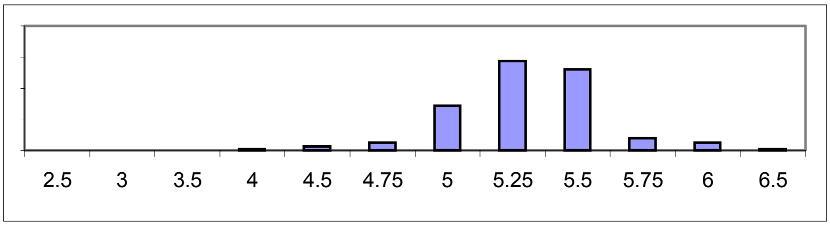

Calving

| | |
|---|---|
| Average | 5.2 |
| Spread (SD) | 0.38 |
| BCS 4.5 and less | 10% |
| BCS 4.75 to 5.25 | 55% |
| BCS 5.5 and more | 35% |




1 month before MSD

| | |
|---|---|
| Average | 5.1 |
| Spread (SD) | 0.39 |
| BCS 4.5 and less | 12% |
| BCS 4.75 to 5.25 | 65% |
| BCS 5.5 and more | 24% |

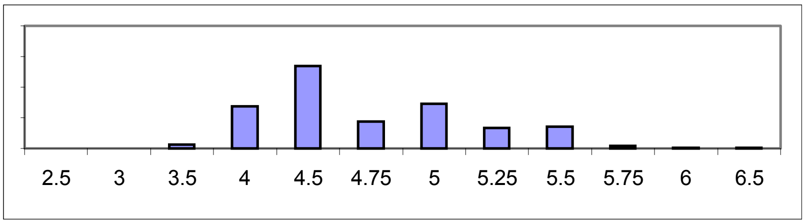



1 month after MSD

| | |
|---|---|
| Average | 5.3 |
| Spread (SD) | 0.30 |
| BCS 4.5 and less | 2% |
| BCS 4.75 to 5.25 | 57% |
| BCS 5.5 and more | 41% |

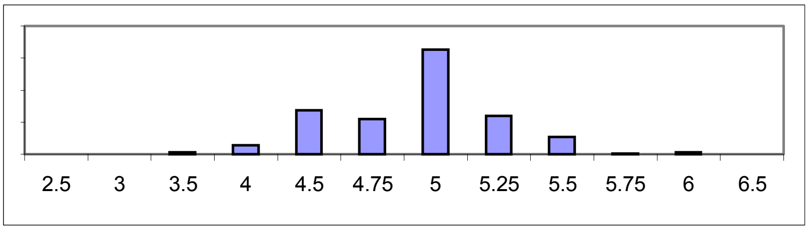
Herd 1 had the greatest peak milk production (28L/cow/day) and also fed the most supplements in early lactation (6 to 7kg cereal grain). The herd maintained its condition from calving through the early lactation period. This herd is a suitable reference herd as it had an average BCS at calving of over 5 and a tight distribution of cows around the average. There was a slight decrease in condition score between calving and the pre-mating visit, but the cows increased BCS from the pre-mating visit to one month after the MSD.

Herd 2



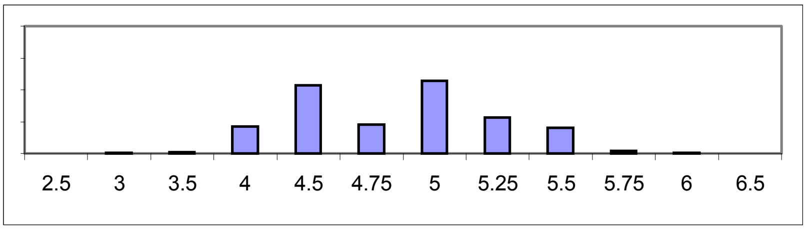

Calving

| | |
|---|---|
| Average | 4.7 |
| Spread (SD) | 0.49 |
| BCS 4.5 and less | 52% |
| BCS 4.75 to 5.25 | 38% |
| BCS 5.5 and more | 10% |




1 month before MSD

| | |
|---|---|
| Average | 4.8 |
| Spread (SD) | 0.45 |
| BCS 4.5 and less | 37% |
| BCS 4.75 to 5.25 | 52% |
| BCS 5.5 and more | 11% |



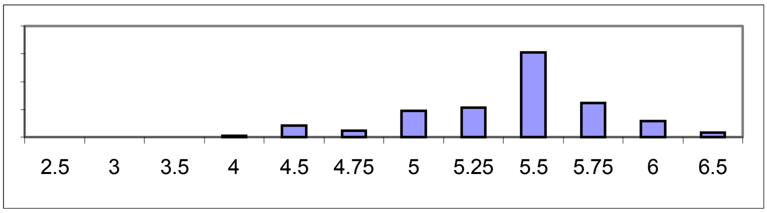

1 month after MSD

| | |
|---|---|
| Average | 4.9 |
| Spread (SD) | 0.36 |
| BCS 4.5 and less | 22% |
| BCS 4.75 to 5.25 | 70% |
| BCS 5.5 and more | 8% |

Herd 2 had the lowest BCS at calving, and a much greater spread than Herd 1. This herd also had the greatest incidence of anoestrus cows before the start of mating and this association is worth further investigation.

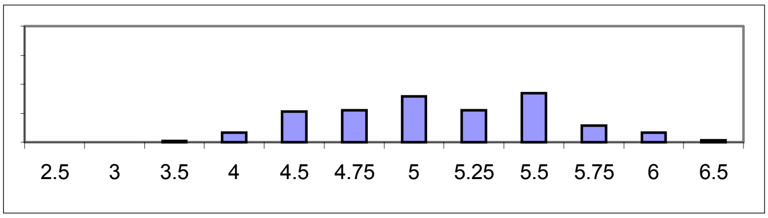
Herd 2 made a concerted effort to increase BCS after calving. This can be seen by the increase in the mean BCS, the reduced spread around the mean, and the proportion of cows with a BCS or less.

Herd 3



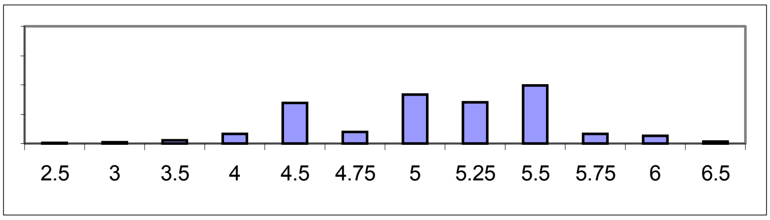

Calving

| | |
|---|---|
| Average | 5.4 |
| Spread (SD) | 0.40 |
| BCS 4.5 and less | 6% |
| BCS 4.75 to 5.25 | 29% |
| BCS 5.5 and more | 65% |




1 month before MSD

| | |
|---|---|
| Average | 5.1 |
| Spread (SD) | 0.50 |
| BCS 4.5 and less | 18% |
| BCS 4.75 to 5.25 | 48% |
| BCS 5.5 and more | 34% |



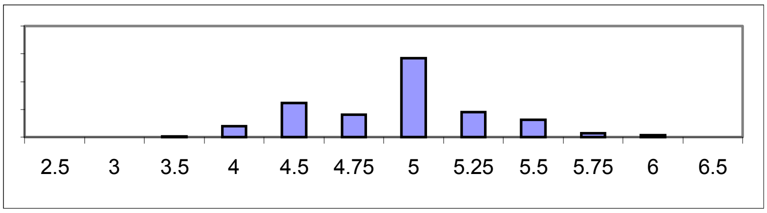

1 month after MSD

| | |
|---|---|
| Average | 5.1 |
| Spread (SD) | 0.55 |
| BCS 4.5 and less | 24% |
| BCS 4.75 to 5.25 | 43% |
| BCS 5.5 and more | 33% |

Herd 3 provided grain in the dairy before calving (lead feeding) which was not done by the other three herds. The result of drying cows off in good condition and then lead feeding was that cows in this Herd calved with a higher BCS than the other herds.

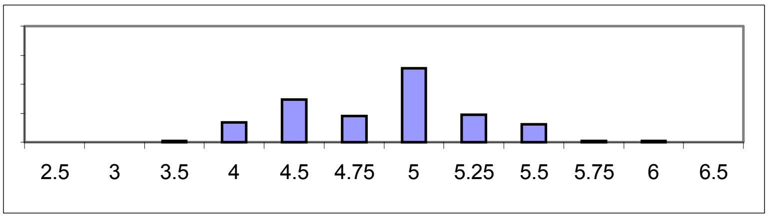
There was a large drop in condition score after calving, as well as in increase in the spread of cows in the herd. The herd maintained this situation through the start of the mating period. This herd fed the least supplements of the four herds.

Herd 4



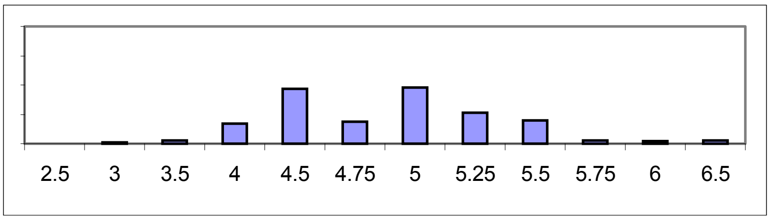

**Calving**

| | |
|---|---|
| Average | 4.9 |
| Spread (SD) | 0.39 |
| BCS 4.5 and less | 23% |
| BCS 4.75 to 5.25 | 65% |
| BCS 5.5 and more | 12% |




**1 month before MSD**

| | |
|---|---|
| Average | 4.9 |
| Spread (SD) | 0.41 |
| BCS 4.5 and less | 30% |
| BCS 4.75 to 5.25 | 60% |
| BCS 5.5 and more | 10% |




**1 month after MSD**

| | |
|---|---|
| Average | 4.8 |
| Spread (SD) | 0.53 |
| BCS 4.5 and less | 36% |
| BCS 4.75 to 5.25 | 50% |
| BCS 5.5 and more | 15% |

Herd 4 had an average profile of condition scores at the time of calving. After calving there was a group of cows that lost condition, and this is evident in both the spread around the mean and also the number of cows with a BCS of 4.5 and less.

It is of concern that there were around one third of cows with a condition score of 4.5 or less at the time of mating.

# Appendices

## *Appendix 1: Preliminary results*

This manuscript is published in the proceedings of the New Zealand Society of Animal Production. Its contents will be presented at the annual conference in Christchurch, New Zealand in June, 2001.

The results of this manuscript are based on the early pregnancy testing and underestimate the real incidence of Phantom cows because they do not include the cows which lost their pregnancy between the early diagnosis and the 13-week diagnosis. The results also were collated before the availability of the milk progesterone data.

**Disclaimer**: This appendix is a private report for stakeholders. It is a draft submission for a public journal. It has not completed the peer-review process and will become public when published in a peer-reviewed manner. This report must not be copied without permission of the authors, and should instead be considered as a draft that will be published in the public domain as soon as is practical.

**Phantom Cows: A major cause of late pregnancies and reproductive wastage in Australian dairy herds.**

**Short Title**: Non-return, non-pregnant dairy cows

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## Abstract

A major cause of delayed pregnancy and reproductive wastage in Australian dairy cattle is the failure of cows to be re-inseminated within 24 days of an unsuccessful insemination. Four commercial herds were observed to determine the incidence of this syndrome. Those cows inseminated in the first three weeks of the mating season (n=721 cows) were observed for a subsequent return to service, and non-return cows were diagnosed for pregnancy. Overall, 45% of cows were confirmed pregnant to first service, 37% returned to service within 24 days, and 19% were diagnosed as non-return, non-pregnant cows (Phantom Cows). The majority of cases of Phantom Cows (89%) were explained as having: a) a CL but not returning for service; b) having an abnormally long return to service (25 to 39 days); or, c) reverting to an anoestrous state. Other cases (<5% incidence) included ovarian cysts, pyometra and uterine adhesions. Repeated diagnoses of pregnancy identified few cases where a pregnancy appeared to have failed (3.3%). The incidence of Phantom Cows was higher for CIDR-treated anoestrous cows than cows synchronised with prostaglandin, and lowest for those cows not treated. These observations form the basis for further study of this syndrome in Australian herds.

**Keywords**: dairy cow; non-return; non-pregnant; reproductive failure.

## Introduction

A study of reproductive performance in commercial Australian dairy herds (the InCalf Project) has identified that a proportion of cows that were retrospectively diagnosed

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as not pregnant to their first insemination were not re-submitted for a second insemination within 24 days. A colloquial term, Phantom Cows, has been assigned to this syndrome (Cavalieri *et. al.*, 2000). They are defined as cows that: a) are inseminated early in the artificial breeding period; b) are not detected in oestrus within 24 days of the first insemination; and c) are retrospectively diagnosed as not pregnant to the first insemination.

The use of Controlled Breeding Programs where cows are synchronised for both an initial oestrus and then resynchronised for a subsequent oestrus resulted in a 13% (388/3009) incidence of Phantom Cows (Cavalieri *et. al.*, 2000). They contributed to 37% of the non-pregnant group (316/844) as measured at the end of the artificial breeding period (47 days).

The InCalf Project reported that submission rates were reduced in the second round of insemination compared with the first round (DRDC, 1999). Preliminary analysis of the InCalf data has determined that 18% (2560/14,424) of cows inseminated early in the artificial breeding period exhibit this Phantom Cow syndrome (J. Morton & D Nation, *pers. comm.*). Only 24% of the Phantom Cows were pregnant at the end of the artificial breeding period, and 55% pregnant to natural mating while 21% remained not pregnant after a 21-week breeding season. Contemporary herdmates that did not conceive to the first insemination but returned to oestrus 18 to 24 days later (n=3804) had 65% conceive to artificial insemination and 26% to natural mating while 9% were not pregnant.

A simple approximation of the incidence of Phantom Cows can be calculated as the difference between the 24-day non-return rate (cows not seen in oestrus by 24 days after insemination) and the conception rate (cows diagnosed pregnant at least 6 weeks after insemination). This incidence was reported at 16% by Cavalieri *et. al.* (2000).

Previous studies with British and German Friesian cows in the 1970's used elevated milk progesterone concentrations on Days 21 and/or 24 post-insemination as an indicator of pregnancy. Of the cows with elevated progesterone concentrations at this time, 8 to 12% were later found (after Day 40) to be not pregnant (Bulman and Lamming, 1977; Pope *et. al.*, 1976, Ball *et. al.*, 1978).

This study described the incidence of Phantom Cows in four herds which had seasonally concentrated calving patterns and where no treatments were applied which could alter the incidence or duration of the return interval following an unsuccessful first insemination. Incidences of Phantom Cows and cows that were confirmed pregnant to first service were examined for associations with herd, age, interval from calving to mating, body condition score at calving, and treatment preceding first insemination.

## Materials and Methods

### Animals

Four commercial herds were involved in the study. All cows in Herd 1 were enrolled (n=196), while a random selection of cows in Herds 2 to 4 (balanced for age and calving to mating start date interval within herd) was enrolled (n=219, n=197 and n=183, respectively). Cows were fed both pasture and a grain-based supplement, and were managed on a commercial basis. They were enrolled at calving and had a body condition score (BCS; 1 to 8 scale; Grainger *et. al.*, 1982) measured within 7 days. They were only excluded from analyses if they were not inseminated within 21 days after the Mating Start Date (MSD), or if they had calved less than 21 days before the MSD.

Oestrus events in the 30 days before the MSD and insemination dates for at least the first 42 days of mating were recorded. Cows that were not detected in oestrus by 8 days before the MSD were examined per rectum. Those diagnosed as anoestrus were treated with a CIDR ™ device (An-CIDR; Genetics Australia, Bacchus Marsh, Australia) for 8 days. A 2 mg injection of oestradiol benzoate (CIDIROL ™ , Genetics Australia, Bacchus Marsh, Australia) was given at the time of CIDR insertion, and a 1mg injection of oestradiol benzoate was given from 24 to 30h after removal of a CIDR insert.

Cows not expected to display oestrus in the first two weeks of mating (based on a 21 day interval after their last observed oestrus) were treated with prostaglandin-$F_{2\alpha}$ (PG; 5ml Lutalyse®, Pharmacia Upjohn, Sydney, Australia) in the first or second week of mating (Macmillan *et. al.*, 1977). Treated cows were allocated to the earliest possible PG group where the interval from their observed heat to the time of injection was at least six days. Those cows expected to return to oestrus during the first two weeks of mating were not treated and were bred on detection of a spontaneous oestrus.

Pregnancy Diagnosis

Cows that were not re-inseminated within 24 days of first service were submitted for a series of pregnancy examinations. Trans-rectal ultrasonography was conducted manually using a 7.5 MHz probe (without the use of an extender) initially from 28 to 34 days post-insemination. Pregnancy was defined as the presence of an observed embryo in a uterine horn. Cows without an observed embryo were re-examined ultrasonographically seven days later. Only those cows with two consecutive negative diagnoses were confirmed as not pregnant to the initial insemination. Abnormal palpable structures (cystic ovaries, pyometra, and substantial uterine adhesions) were also recorded at the time of diagnoses. At the second diagnoses, those cows that were confirmed not pregnant to the first insemination were also examined to identify whether a corpus luteum was present on one of their ovaries.

Statistical analyses

Each cow was initially categorised according to the success/failure of the first insemination and subsequent return. The three categories were pregnant to the first insemination, returned to oestrus within 24 days of the first insemination, and non-pregnant, non-return within 24 days of first insemination (Phantom Cows).

Phantom cows were categorised as having a late return to service (25 to 39 days post-insemination; Long Return); no return to service within 39 days but having a CL on one or both ovaries (No Return); no return to service within 39 days and having no CL on either ovary (Anoestrus); a large follicle on one ovary (≥25mm antral follicle diameter; Cyst); an open pyometra infection where pus was observed in the uterine horn via ultrasonography as well as manual removal from the vagina (Pyometra); and substantial adhesions between the uterus and the rumen (Adhesions).

Explanatory variables were categorised for herd, age (2yo, 3yo, 4-6yo, 7yo or older), calving to MSD interval (4-6 weeks, 7-9 weeks, 10-12 weeks, 13+ weeks), BCS at calving ($\leq$ 4.5, 4.75 to 5.25, or $\geq$ 5.5) and the physiological state before the first service (An-CIDR, PG or No Treatment).

Logistic regression was performed in a backwards stepwise manner by fitting significant variables from a univariate model (at $p<0.25$) to a multivariate model. Each model compared a single category (such as Phantom Cows) with the rest of the study population. Interactions were evaluated for those terms that were significant ($p<0.05$) in the multivariate model. No interactions were significant ($p>0.1$), thus only main effects that were significant (p<0.05) in the final multivariate model have been reported.

## Results

### Animals

The number of cows included in the study (enrolled cows minus excluded cows) are reported in Table 1, and categorised according to the physiological state before the first service. The return-to-service intervals are reported in Figure 1. Return intervals were measured up to 39 days post-insemination, and those cows that had not returned to service were then described as either pregnant, or as cows with no return to service.

### Incidence of Pregnant and Phantom cows

The incidence of cows that were confirmed pregnant to first service, returned for a second service within 24 days, or were Phantom Cows is reported in Table 2. Crude associations with herd, age, interval from calving to MSD, BCS at calving and physiological state before the first service are described. Significant associations from multivariate analyses are highlighted (bold) in the table. There was a significant relationship between the incidence of cows pregnant to first service and a herd effect as well as the physiological state before the first oestrus (Table 2). Cows selected for the An-CIDR treatment had a higher incidence of the Phantom Cow syndrome than PG treated cows, which in turn, had a higher incidence than no treatment (p<0.05, Table 2).

### Subcategories of Phantom Cows

Of the subcategories, 89% of cases were described as Long Return, No Return or Anoestrus (Table 3). The proportion of Phantom Cows which were Long Return cows was associated with herd and physiological state before the first service ($p<0.05$), No Return cows were associated with physiological state before the first service ($p<0.05$), and Anoestrous cows were associated with age, physiological state before the first service ($p<0.05$) and also had an increased tendency among cows calving with a BCS $\leq 4.5$ ($p<0.10$; Table 3).

### Diagnoses of Pregnancy

The differential diagnosis of pregnancy was precise after excluding the gross abnormal cases of ovarian cysts, pyometra and adhesions. All pregnant cases (n=321) had at least 15mm (diameter) of fluid in the uterine lumen and an embryo ≥5mm in length. There were only 11 cases (3.3%) that appeared to have lost a pregnancy. Sequential observations of these cases revealed an accumulation of cloudy fluid and flaccid embryonic membranes. In some of these cases, an indistinguishable mass that may have been a resorbing embryo was observed. These 11 cases had not returned to oestrus within 39 days of insemination and were categorised as Phantom Cows with No Return.

## Discussion

It is of major concern to the Australian dairy industry that 19% of the study population were not confirmed pregnant to the first insemination, and had not returned for a second service within 24 days. This study has described the incidence of these Phantom Cows, and broken down this syndrome into selected sub-categories. This is the first report to describe subcategories of the Phantom Cow syndrome, and to investigate associations with age, interval from calving to MSD, BCS at calving, and intervention according to the physiological state before the first service.

A potential cause of the Phantom Cow Syndrome is the failure to detect a return to oestrus. These cases would be included in the No Return subcategory as they will have a CL on an ovary but will not have been detected in oestrus. This subcategory is most likely to represent the maximum incidence of failure to detect a subsequent oestrus. It is of note that the proportion of Phantom Cows which were No Return cows is lower amongst the CIDR-treated anoestrous cows, indicating that failure to detect oestrus is not the predominant cause of Phantom Cows for this group.

The cows that had a return to service interval between 25 and 39 days (Long Return) may have abnormal luteal function. This has been observed in postpartum cows (Ball and McEwen, 1998), but there is no comparable evidence reported for inseminated cows. Within the Phantom Cow population, Long Returns were more common in cows in the An-CIDR and No Treatment groups than the PG treatment group. While the occurrence of a prolonged oestrous cycle following a period of anoestrus (as induced in the An-CIDR group) may be similar to other postpartum observations (Ball and McEwen, 1998), the higher incidence in cows with no treatment compared with cows that had PG treatment is unexplained and has not been previously reported.

Anoestrous cows detected following the first insemination were most common in the An-CIDR group. The re-occurrence of anoestrus after the first insemination in the An-CIDR group has been reported previously (Rhodes *et. al.*, 1999). The anoestrous state implies that the original treatment did not successfully induce the resumption of a regular oestrous cycle. It is possible that ovulation did not occur after the initial anoestrous treatment. The greater incidence of anoestrus in younger cows, and those calving in a low BCS concurs with previous studies of factors affecting the post-partum anovulatory interval (McDougall, 1994)

The occasional diagnosis of anoestrus after first insemination of cows in the PG and No Treatment groups indicated that there may still be a proportion of these populations that had been observed in oestrus and inseminated but resumed an anoestrous state. Alternatively, they may have been chronically anoestrus and had a first insemination to a false oestrus (Rhodes *et. al.* 1999). This false oestrus scenario is not likely as these cows must have been observed in oestrus in the pre-mating period to be excluded from the anoestrous treatment, as well as having to be observed in oestrus a second time after the MSD and inseminated.

In conclusion, Phantom Cows are common and can be categorised into three physiological categories: 1) cows that fail to return to service within 39 days after insemination but have a CL present on an ovary; 2) cows with a long return, 25 to 39 days after the first insemination; and 3) anoestrous cows with no CL on either ovaries. Gross abnormalities, namely ovarian cysts, pyometra and uterine adhesions, as well as pregnancy failure were only minor contributors to the Phantom Cow syndrome. This study has demonstrated that the Phantom Cow Syndrome is more likely to occur in anoestrous cows treated with a CIDR insert, less likely to occur in cows selected for PG treatment, and least likely to occur in untreated cows.

## Acknowledgements

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**Table 1:** Number of cows observed in each herd (after exclusions), according to treatment before the first insemination.

| Treatment | Herd 1 | 2 | 3 | 4 | Total |
|---|---|---|---|---|---|
| CIDR | 60 | 97 | 33 | 40 | 230 |
| PG | 59 | 56 | 96 | 69 | 280 |
| No treatment | 50 | 55 | 51 | 55 | 211 |
| Total | 169 | 208 | 180 | 164 | 721 |

**Table 2:** The incidence of cows that conceive to first service (Pregnant Cows), that return to oestrus within 24 days of service (Returned to service) and non-return, non-pregnant cows (Phantom Cows). Those associations that are significant in the final multivariate model are in bold (p<0.05).

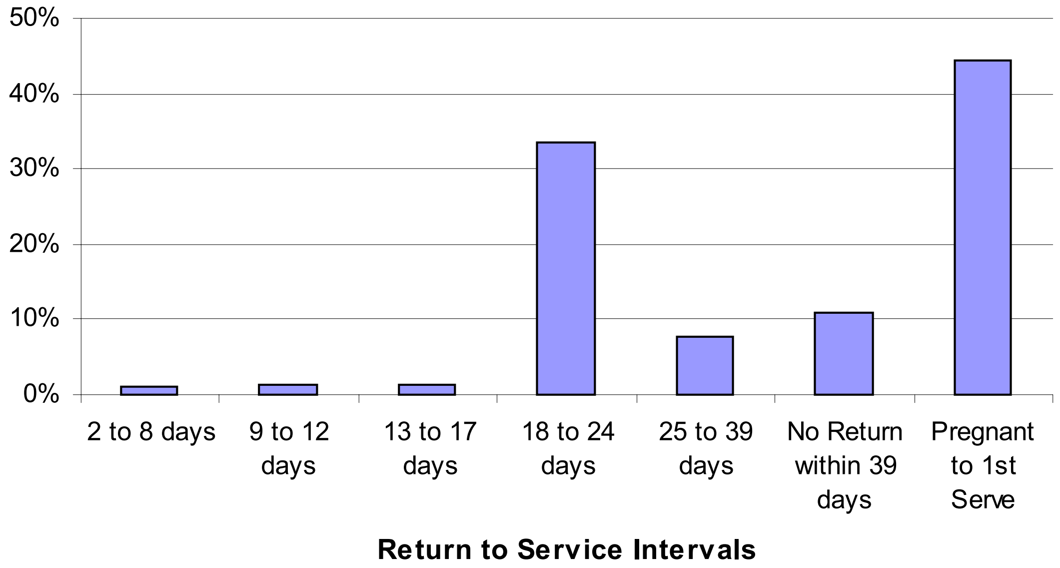
| | n | Pregnant Cows | Returned to Service | Phantom Cows |
|---|---|---|---|---|
| Overall | 721 | 321 (45%) | 264 (37%) | 136 (19%) |
| **Association with herd** | | | | |
| Herd 1 | 169 | **76 (45%)** | 58 (34%) | 35 (21%) |
| Herd 2 | 208 | **78 (38%)** | 89 (43%) | 41 (20%) |
| Herd 3 | 180 | **109 (61%)** | 39 (22%) | 32 (18%) |
| Herd 4 | 164 | **58 (35%)** | 78 (48%) | 28 (17%) |
| **Association with age** | | | | |
| 2yo | 130 | 58 (45%) | 53 (41%) | 19 (15%) |
| 3yo | 109 | 55 (50%) | 34 (31%) | 20 (18%) |
| 4-6yo | 279 | 129 (46%) | 97 (35%) | 53 (19%) |
| 7+yo | 203 | 79 (39%) | 80 (39%) | 44 (22%) |
| **Association with calving to MSD Interval** | | | | |
| 4 to 6 weeks | 61 | 20 (33%) | 24 (39%) | 17 (28%) |
| 7 to 9 weeks | 198 | 80 (40%) | 69 (35%) | 49 (25%) |
| 10 to 12 weeks | 379 | 179 (47%) | 142 (37%) | 58 (15%) |
| 13+ weeks | 81 | 42 (52%) | 28 (35%) | 11 (14%) |
| **Association with Body Condition Score at Calving** | | | | |
| ≤4.5 | 178 | 68 (38%) | 76 (43%) | 34 (19%) |
| 4.75 to 5.25 | 317 | 135 (43%) | 122 (38%) | 60 (19%) |
| ≥5.5 | 211 | 111 (53%) | 64 (30%) | 36 (17%) |
| **Association with treatment according to physiological state before first service** | | | | |
| An-CIDR Treatment | 230 | **75 (33%)** | 91 (40%) | **64 (28%)** |
| PG Treatment | 280 | **141 (50%)** | 94 (34%) | **45 (16%)** |
| No Treatment | 211 | **105 (50%)** | 79 (37%) | **27 (13%)** |

**Table 3:** The incidence of Phantom Cows that have ultrasonographic evidence of a CL on either ovary but have not returned to service (No Return), cows that have returned to service 25 to 39 days after insemination (Long Return), or cows with no CL on either ovary and have not returned to service (Anoestrus). Those associations that are significant in the final multivariate model are in bold ($p<0.05$).

| | n * | No Return | Long Return | Anoestrus |
|---|---|---|---|---|
| Overall | 136 | 48 (35%) | 53 (39%) | 23 (17%) |
| **Herd Effect** | | | | |
| Herd 1 | 35 | 7 (20%) | **20 (57%)** | 3 (9%) |
| Herd 2 | 41 | 17 (41%) | **13 (32%)** | 8 (20%) |
| Herd 3 | 32 | 9 (28%) | **15 (47%)** | 7 (22%) |
| Herd 4 | 28 | 15 (55%) | **5 (18%)** | 5 (18%) |
| **Age Effect** | | | | |
| 2yo | 19 | 5 (26%) | 9 (47%) | **5 (26%)** |
| 3yo | 20 | 2 (10%) | 10 (50%) | **7 (35%)** |
| 4-6yo | 53 | 23 (43%) | 20 (38%) | **8 (15%)** |
| 7+yo | 44 | 18 (41%) | 14 (32%) | **3 (7%)** |
| **Calving to MSD Interval** | | | | |
| 4 to 6 weeks | 17 | 4 (24%) | 7 (41%) | 5 (29%) |
| 7 to 9 weeks | 49 | 13 (27%) | 22 (45%) | 12 (24%) |
| 10 to 12 weeks | 58 | 24 (41%) | 20 (34%) | 6 (10%) |
| 13+ weeks | 11 | 6 (55%) | 4 (36%) | 0 |
| **Body Condition Score at Calving** | | | | |
| ≤4.5 | 34 | 9 (26%) | 11 (32%) | 11 (32%) |
| 4.75 to 5.25 | 60 | 26 (43%) | 20 (33%) | 6 (10%) |
| ≥5.5 | 36 | 10 (28%) | 20 (56%) | 5 (14%) |
| **Treatment according to physiological state before first service** | | | | |
| An-CIDR Treatment | 64 | **9 (14%)** | **27 (42%)** | **18 (28%)** |
| PG Treatment | 45 | **28 (62%)** | **12 (27%)** | **3 (7%)** |
| No Treatment | 27 | **11 (41%)** | **14 (52%)** | **2 (7%)** |

* Cows with a cyst (n=7), pyometra (n=4), or adhesions (n=1) have not been included in the table but are included all calculations of proportions of the Phantom Cow population.

**Figure 1:** The return to service interval for the study population. Cows which had not returned to service within 39 days were categorised as either pregnant or as having no return to service.



## *Appendix 2: Early pregnancy diagnosis*

This manuscript will be submitted to the Australian Veterinary Journal. This manuscript provides the industry with a reference of the accuracy of early pregnancy diagnosis with an ultrasound device. The accuracy of such a diagnoses may be a powerful tool in identifying problem cows and deciding on appropriate treatment.

# Accuracy of bovine pregnancy diagnosis via trans-rectal ultrasonography at 28 to 35 days post-insemination.

D.P. NATION [a], J. MALMO [b], G.M. DAVIS [b] and K.L. MACMILLAN [a]

**Objective** To evaluate the use of real-time ultrasonography to diagnose pregnancy at 28 to 35 days post-insemination in dairy cows.

**Methods** Cows that did not return to oestrus between 18 and 24 days after a first insemination (n=526) were examined by trans-rectal ultrasonography from 28 to 35 days post-insemination. Pregnancy was confirmed by the observation of a foetus, but fluid in the uterine horn and the presence of embryonic membranes were also noted. Where pregnancy was not confirmed by the observation of a foetus, a second diagnosis, 7 days later, confirmed these remaining cows as pregnant or not pregnant to the first insemination. Pregnancy status at this early diagnoses was compared with a manual transrectal pregnancy diagnoses performed 10 to 13 weeks post-insemination (13-week examination).

**Results** There were 44% of cows that were pregnant to the first service, 34% that had returned for a second service 18 to 24 days after the first insemination, and 20% of cows that were not pregnant, and had not returned normally for a second service (non-pregnant, non return) within 24 days of their initial insemination. The presence of a foetus at 28 to 35 days post-insemination can be accurately estimated by a simplified method where uterine fluid accumulation and embryonic membranes were observed. Foetal loss between the early diagnosis and the 13-week examination (9% of pregnancies) indicated that 28 to 35 days post insemination was too early to obtain a reliable measure of pregnancy status.

**Conclusion** Early pregnancy diagnosis with trans-rectal ultrasonography is an accurate tool to identify non-pregnant, non-return cows. Diagnosis can be simplified by the observation of uterine fluid accumulation and embryonic membranes, as opposed to the more involved process of observing the foetus.

Key words: Pregnancy diagnosis, bovine, ultrasonography

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CL Corpus luteum

MSD Mating Start Date

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While there have been many reports of ultrasound use for early pregnancy diagnosis in Europe and the USA [1-5], the commercial uptake of this technology has been poor in

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Australia. The commercial potential of such a service might be improved if an indirect form of pregnancy diagnosis, i.e. the observation of uterine fluid accumulation and embryonic membranes, can be substituted for the requirement to observe a foetus. It would be expected that a foetal observation diagnosis would be more definitive, and the simplified diagnosis compromised by the occasional presence of a uterine infection. Scenzi *et. al.* described a similar comparison where the direct observation of a foetus was more accurate then assays for the presence of pregnancy specific proteins in plasma, but resulted in more false negative diagnoses. [6]

The ability to diagnose pregnancy as early as possible after an insemination is of importance due to the significant population of modern Holstein dairy cows which are not pregnant to the first insemination and do not return for a subsequent service within 24 days. Non-pregnant, non-return cows comprised 19% of a study population in Australian dairy herds. [7]

This study serially observed inseminated cows from 28 days post insemination via trans-rectal ultrasonography. A simplified pregnancy diagnosis, as defined by the presence of uterine fluid accumulation and the presence of embryonic membranes at a single ultrasonographic observation, was compared against the standards of both a) the observation of a foetus from serial ultrasonographic observations; and b) manual palpation from 10 to 13 weeks post-insemination.

## Materials and methods

### *Animals*

A sub-population was selected from four commercial, Australian dairy herds. Cows (n=777; n=169 to n=240 cows per herd) were randomly selected from the whole herd, balanced for age as well as the interval from calving to the MSD. Those cows which had not returned to oestrus 18 to 24 days post-insemination (n=526) were selected for serial pregnancy diagnosis. All cows in the four herds were examined from 10 to 13 weeks post-insemination, via rectal palpation, to diagnose the pregnancy status (13-week examination). The pregnancy status of herdmates from three of the four herds (n=146 to 178 cows per herd) was compared with the study population at this time.

*Early pregnancy diagnosis*

An Aloka 500 ultrasound machine with a 7.5MHz probe was used to visualise the entire length of both uterine horns and, where necessary, ovarian structures. The ultrasound probe was carried into the rectum by the operator and moved over both uterine horns so that the appropriate structures could be visualised. The ultrasound image was observed by both the senior author of the paper and by the ultrasound operator and the confirmation of a foetus was only made with agreement between both observers.

Each herd was visited weekly, selecting groups of cows that had been inseminated 28 to 35 days previous. Observation of both uterine horns was described by the side of fluid accumulation, the maximum observed diameter of uterine fluid (lumen diameter in 5mm intervals), the presence of embryonic membranes, the presence of a foetus, the maximum length of the foetus, and the presence of a heartbeat. Abnormal palpable structures were also recorded. These included an enlarged ovary due to a follicle ≥25mm in diameter (Cystic Ovary), substantial uterine adhesions (Adhesions), and open pyometra that was diagnosed as both a grainy image of fluid in the uterine lumen and pus manually removed from the vagina (Pyometra).

Diagnosis was definitive where an abnormality was recorded or a foetus was observed. Those cows where a foetus was not observed, and where no other abnormality was detected, were re-examined at the next weekly visit to confirm the pregnancy status of the cow. Serial observation also determined additional abnormal categories that comprised a) the abnormal small size of both the foetus and uterine lumen, with no heartbeat evident (Small Foetus); and b) evidence of a lost pregnancy with substantial cloudy uterine fluid, flaccid membrane-like structures, and in some cases a white echogenic body which may be a resorbing foetus (Lost Pregnancy?).

*Statistical Analysis*

Cows were classified according to their observation during serial ultrasonographic examinations. Abnormal cases were then excluded from further analyses. Cows were categorised according to the accuracy of diagnosing uterine fluid and embryonic membranes at the first examination (Simplified pregnancy diagnosis) compared with a) the observation of a foetus from serial diagnoses (Foetal observation); or b) the manual diagnosis of pregnancy six weeks after the end of the artificial breeding period (13-week examination).

## Results

Categories from serial ultrasonographic pregnancy diagnoses are presented in Table 1. Of the study population (n=777), 44% were pregnant to the first service, 3% had observed abnormalities, 34% returned for a second service within 24 days of the first service, and 20% were not pregnant and had not returned to service within 24 days.

A comparison of accuracy parameters between a simplified diagnosis and foetal observation is presented in Table 2. All accuracy parameters were over 90%, with the least accuracy in the negative predictive value (92%).

Both the Simplified pregnancy diagnosis and Foetal Observation have similar accuracy when compared to the 13-week examination (Table 3). Both methods have reduced accuracy due to the pregnancy loss between the early diagnoses and the 13-week examination (31 cases, or 9% pregnancy loss). There were also 2 cases where cows were reclassified from not pregnant to pregnant that could be attributed to an error in manual palpation or a missed ultrasound diagnosis (1% error).

The conception rate to first service, as calculated from the Mid-gestation diagnosis, did not differ between the study population and their herdmates (Table 4). This indicates that there was not a deleterious effect of ultrasonographic examination on the establishment of pregnancy.

Of all the pregnant cows observed with serial ultrasonography, 58% were pregnant in the right uterine horn. The changes in embryo size, uterine horn diameter and the presence of a heartbeat are described in Figure 1. The length of the embryo increased from an average of $6.9 \pm 0.20$mm (sem) at 28 days post-insemination to $13.5 \pm 0.34$mm at 35 days post-insemination (Figure 1a). Over this period the proportion of pregnant cows with a lumen diameter ≥25mm increased from 0% to 85% (Figure 1b), and the proportion of embryos with an observed heartbeat increased from 67% to 100% (Figure 1c).

## Discussion

The use of a simplified pregnancy diagnosis (with an observation of at least 15mm fluid in the uterine horn as well as embryonic membranes) was an accurate estimation of the presence of an embryo at the time of observation. However, there was a substantial loss of pregnancy between the early ultrasonographic observation and a mid-gestation diagnosis, resulting in the simplified pregnancy diagnosis being a less reliable predictor of pregnancy status at mid-gestation.

These observations are reliant on a systematic observation of the entire length of both uterine horns and the conclusions made are thus dependent on operator ability. Some cases involved persistence to follow the uterine horn through contortions and also at times where it protruded cranial to the pelvic floor. The identification of fluid and membranes is a more efficient process than the time required to find an embryo as small as 6mm in a convoluted uterine horn. As such, this simplified pregnancy diagnosis method provides for a rapid gain in efficiency in the diagnosis of pregnancy at this early stage.

The loss of pregnancy between 5 weeks gestation (early diagnosis) and 10 to 13 weeks gestation (mid-gestation diagnosis) of 9% of cows confirmed pregnant corresponds to a pregnancy loss in the whole study population of 3.5%. This is similar to previous reports of losses between 30 days and term of 4% to 6%. [8-10] The pregnancy loss observed in this study is unlikely to be caused by the intervention with serial ultrasonography (Table 3), which concurs with a previous report. [9]

The simplified early pregnancy diagnosis had a consistent negative predictive value of 92 to 93% as compared to the Foetus observation and the Mid-gestation observation. This method provides a suitable diagnoses of non-return, non-pregnant cows that could be treated with a progesterone-based synchrony protocol. The 8% inaccuracy contraindicates the use of prostaglandin in such a protocol due to the chance of a false diagnosis of non-pregnancy.

In conclusion, early pregnancy diagnosis from 28 to 35 days after insemination is possible in commercial Australian conditions. A simplified diagnosis where substantial uterine fluid and embryonic membranes are observed is a suitable compromise to the effort required to observe a foetus. However, there were 9% of foetuses lost between the early diagnoses and a mid-gestation diagnoses, so an early diagnosis is not a reliable indicator of the pregnancy status at mid-gestation, and thus at term. The value of such an early technique is an accurate diagnosis of non-pregnancy which is of merit due to the incidence of cows that are not pregnant to the first service and do not return for a second service within 24 days.

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Table 1. The diagnoses of pregnancy status and abnormalities from serial ultrasonography commencing from 28 to 35 days post-insemination.

| Description | No. of cases |
|---|---|
| Foetus observed at 1st observation | 289 |
| Uterine fluid and embryonic membranes observed at 1st diagnoses, pregnancy confirmed from subsequent observation of foetus | 40 |
| No evidence of gravid structures at the 1st diagnoses, and a foetus was observed at a subsequent diagnoses | 13 |
| Uterine fluid and embryonic membranes observed at 1st diagnoses, no foetus was observed at subsequent diagnoses | 4 |
| No evidence of gravid structures at two consecutive diagnoses | 151 |
| Abnormal embryo observed | 2 |
| Lost Pregnancy? | 11 |
| Cystic ovary | 9 |
| Pyometra | 6 |
| Adhesions | 1 |

Table 2. Accuracy parameters for the comparison of a Simplified pregnancy diagnosis (the observation of ≥15mm fluid in the uterine lumen and embryonic membranes at a single pregnancy diagnosis) with Foetal observation (observation of a foetus during serial ultrasonographic diagnoses.

| | No. of cases |
|---|---|
| Correct Positive (a) | 329 |
| Correct Negative (b) | 151 |
| False Positive (c) | 4 |
| False Negative (d) | 13 |
| | |
| Sensitivity (a / a + d) | 96% |
| Specificity (b / b + c) | 97% [a] |
| Positive Predictive Value (a / a + c) | 99% [c] |
| Negative Predictive Value (b / b + d) | 92% |

a,b c,d: Refer to significant differences ($p<0.05$) from a chi-square analyses

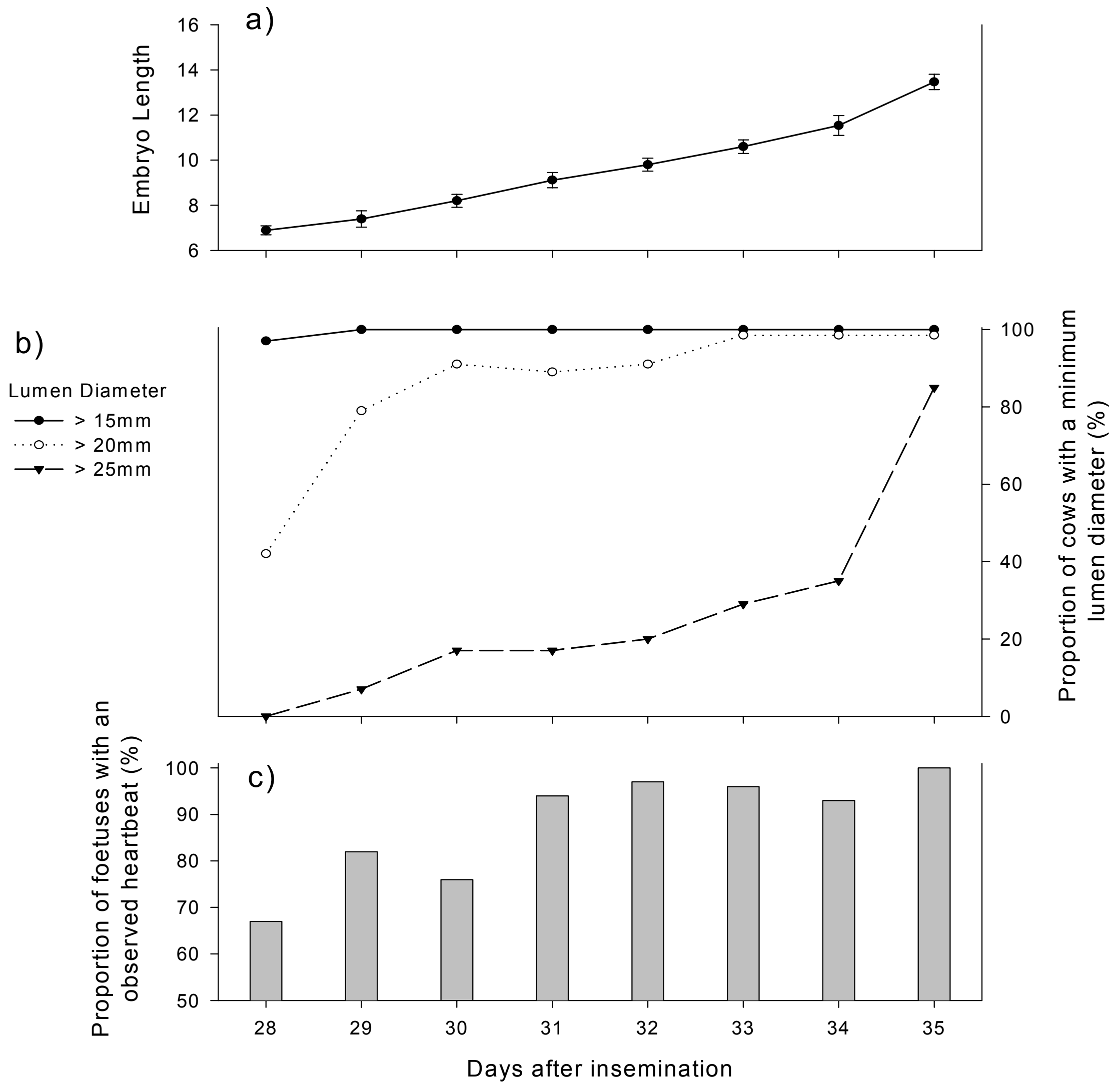
Table 3. Accuracy parameters for the comparison of either a Simplified pregnancy diagnosis (the observation of ≥15mm fluid in the uterine lumen and embryonic membranes at a single pregnancy diagnosis) or Foetal observation (observation of a foetus during serial ultrasonographic diagnoses) with a 13-week examination.

| | Simplified diagnoses | Foetal observation |
|---|---|---|
| Correct Positive (a) | 301 | 305 |
| Correct Negative (b) | 151 | 155 |
| False Positive (c) | 31 | 26 |
| False Negative (d) | 14 | 11 |
| | | |
| Sensitivity (a / a + d) | 96% | 97% |
| Specificity (b / b + c) | 83% | 86% |
| Positive Predictive Value (a / a + c) | 91% | 92% |
| Negative Predictive Value (b / b + d) | 92% | 93% |

Table 4. The comparison of conception rate to first service (where the first service occurred in the 3 weeks following MSD) for the study population and their contemporary herdmates at the Mid-gestation examination.

| | Study population | Herdmates |
|---|---|---|
| Overall | 43% | 40% |
| | | |
| Herd 1 | 39% | 37% |
| Herd 2 | 35% | 38% |
| Herd 3 | 57% | 46% |

Figure 1. The change in gravid parameters from 28 to 35 days post-insemination. Parameters include a) the length of the foetus (average ± sem); b) the proportion of the pregnant population with a uterine horn diameter greater than 15, 20 or 25mm; and c) the proportion of foetuses observed with a heartbeat.



## *Appendix 3: Risk factors of Phantom Cows in the InCalf database*

This manuscript is a collaboration with Dr John Morton and the InCalf Project. It is a powerful study with 14,424 cows from 114 herds. This has allowed for a confident prediction of risk factors for Phantom Cows. Many variables were tested, but there were also some notable exceptions, such as body condition score.

**Disclaimer**: This appendix is a private report for stakeholders. It is a draft submission for a public journal. It has not completed the peer-review process and will become public when published in a peer-reviewed manner. This report must not be copied without permission of the authors, and should instead be considered as a draft that will be published in the public domain as soon as is practical.

# Cow level risk factors associated with non-pregnant, non-return dairy cows.

D.P. Nation [a], J. Morton [b] and K.L. Macmillan [a]

## Abstract

The risk factors associated with cows not pregnant to the first service, and not returning for a second service within 24 days, were analysed from records of 14,424 cows in 114 herds. These non-pregnant, non-return cows were compared with a) cows that are pregnant to the first service; and b) cows which do not conceive to the first service and return to oestrus 18 to 24 days later by backwards, stepwise logistic regression. Non-pregnant, non-return cows comprised 18% of the study population of inseminated cows. The six variables that were included in each of the final models were the interval from calving to MSD, the protein composition of milk in the first 120 days of lactation, age, retained foetal membranes, diagnosis and treatment with synchrony drugs, and herd. These cow-level risk factors may be used to determine at-risk cows for early veterinary intervention.

Key words: logistic regression, cattle – reproductive performance

## Introduction

There is a significant population of dairy cows in Australia that are inseminated, do not conceive to this insemination, and do not return for a second service within 24 days. The incidence has been reported between 13% and 19% of inseminated dairy cows (Cavalieri *et. al.*, 2000; Nation *et. al.* 2001b).

The presence of these non-pregnant, non-return (NRNP) cows may be attributed to a number of causes including embryo mortality, abnormal luteal duration and function, or oestrus detection errors. These putative causes result in the need to compare the

NRNP cows to both a) cows that were pregnant to the first insemination (Pregnant); and b) cows that were not pregnant to the first insemination and returned to service 18 to 24 days later (Return24). The former comparison is meaningful to determine risk factors associated with the failure to establish a pregnancy, and the latter comparison is meaningful to determine risk factors associated with the failure to be detected for a second oestrus within 24 days. The aim of this study is to assess selected cow level factors that may increase the incidence of NRNP cows relative to the population of Pregnant or Return24 cows.

## Materials and methods

### Study sample

A large field experiment (DRDC, 2000) described the data collection and reproductive performance of 24,833 cows in 124 herds that had a seasonally concentrated calving pattern. The records of cows enrolled in this field experiment were used to screen cows suitable for this study. Cows were excluded from this data set according to Table 1. The resultant dataset comprised 14,424 cows from 114 herds.

### Definition of the outcome variable

The enrolled cows were then categorised according to a) a successful pregnancy to the first service (Pregnant; n=7129); b) cows that were not pregnant to the first service but returned for a second service 18 to 24 days later (Return24; n=3804); c) cows that were not pregnant to the first service but returned for a second service 2 to 17 days later (Return17; n=931); and d) cows that were not pregnant to the first service and did not return to service within 24 days (NRNP; n=2560). Of the cows that were served during the artificial breeding period, 49.4% were Pregnant, 26.4% were Return24, 6.5% were Return17 and 17.7% were NRNP.

### Definition of explanatory variables

The variables under study are described in Table 2. The decision to analyse an explanatory variable in a continuous or categorical nature was made on the basis of the linearity of a plot of the b-estimate against the median for each quartile. Variables were considered to be continuous when the plot neared linearity.

The variables under study were selected from a preliminary screening to minimise the chance of collinearity. Collinear variables were only identified in the milk production series, and in each case the variable selected was of a more primary derivation and more likely to be of interest in the final outcome.

Statistical procedure

Two separate analyses were conducted to determine the incidence of a) NRNP cows relative to Pregnant cows( NRNP:Pregnant); and b) NRNP cows relative to Return24 cows (NRNP:Return24).

For each model, cow level factors were analysed by logistic regression in the SAS software program (PROC GENMOD, SAS institute Inc., 2000) in a bivariate model with herd as the constant second variable. Only factors associated (likelihood-ratio $\chi^2$-test, $p<0.25$) with the incidence of NRNP cows were offered to the multivariate model (Table 2).

A binomial logistic regression model was performed with SPSS (V9.5) using a backwards stepwise approach that removed the variable with the highest p-value on the basis of a likelihood-ratio $\chi^2$-test. The model ran automatically until it determined a final model where all factors were significant at $p<0.05$ (two-tailed). Odds ratios and their 95% confidence intervals were presented. Interaction terms between variables were not tested.

## Results

Of the 28 variables tested, only eight were included in the final model of NRNP:Pregnant and six were included in the final model of NRNP:Return24 (Table 3). The five variables common to both analyses were herd, interval from calving to MSD, average protein composition in the first 120 days of lactation, retained foetal membranes and age. Additional variables in the final NRNP:Pregnant model were certainty of insemination, vaginal discharge, and carried over, while the semen used (sire) was included in the final NRNP:Return24 model.

There was large variation between herds and semen used (sire) that was not explained by other factors in the final model. The distribution of odds ratios for each herd was similar in both analyses with only the NRNP:Pregnant model reported (Figure 1). The reference herd had the average incidence of NRNP cows and had over 200 cases. The odds ratio for each sire of the semen used is presented in Figure 2. The reference level was the pooled group of miscellaneous sires.

## Discussion

This study is the first to determine cow level risk factors for the incidence of non-return, non-pregnant cows compared with those cows that either were pregnant to the first service or returned to service 18 to 24 days after the first service. It is of interest that six variables were included in both final models.

The definition of each category was made retrospectively, and at a time when it was too late to avoid the economic setbacks associated with non-pregnant, non-return cows. The identification of these six risk factors may provide herd managers a selective tool to further investigate non-return cows. If a cow fits an at-risk profile then it would be wise not to assume that cows are pregnant if they have not returned to oestrus. It has been shown that non-pregnancy can be detected accurately in commercial Australian conditions from 28 days post-insemination (Nation *et. al.* 2001a).

The inverse relationship between the incidence of NRNP cows and the interval from calving to the mating start date (MSD) is consistent with previous reports of a similar relationship with the proportion of the herd pregnant at 6 weeks, the proportion of cows bred within 3 weeks, and the conception rate to the first insemination (DRDC, 2000). There is a period of anovulatory anoestrus after parturition and a reduced calving to MSD interval increases the incidence of anoestrus cows at the start of mating (McDougall *et. al.,* 1998). The increased incidence of NRNP cases in 2 year old cows may be associated with their increased likelihood of being anoestrus (McDougall *et. al.*, 1998), but this relationship has not been established in older cows. The increased incidence of NRNP cases in older cows may be associated with a greater incidence of embryo mortality or compromised luteal function (Zavy, 1994).

The delay in pregnancy associated with retained foetal membranes and vaginal discharges has been reported previously (Erb and Martin, 1981). This paper is the first to relate these conditions to an increased incidence of NRNP cases. It is of interest that other calving descriptions i.e. induced parturition, twins, abortion and dystocia were not included in the final models.

The relationship between the protein proportion of early lactation milk (120 days) and the incidence of NRNP cases has not been reported in peer-reviewed journals. A report on the same dataset (DRDC, 2000) described a similar relationship between the proportion of protein in milk and the herd pregnant at 6 weeks, reduced proportion of cows bred within 3 weeks, and the conception rate to the first insemination. As this relationship represents changing protein proportions within herds, it is less likely that it could be attributed to management and environmental factors, and more likely that it is attributed to genetic differences.

There was no observed effect of a prostaglandin based synchrony protocol on the incidence of NRNP cows as compared to cows with no recorded use of synchrony drugs. There was, however, and increased likelihood of NRNP cases for cows that were diagnosed as anoestrus before the first insemination (no palpable corpus luteum or record of an oestrus event) and treated with a progesterone based protocol. This increased likelihood could not be separated to investigate the effect of either the physiological state, or the progesterone based protocol, and therefore these factors must be considered together.

The inclusion of herd in the final model represents the large degree of variation that could not be explained by the other explanatory variables. The large range of incidence in herds (Figure 1) adjusted for the other variables in the final model demonstrates the potential reduction in the incidence of NRNP cases if the causes and appropriate preventative methods or treatments could be developed.

The increased incidence of NRNP cases in cows carried over for two years is consistent with their inability to sustain a pregnancy over the past two years. These cows are of inherently lower fertility. It is of interest that cows that have been inseminated in a compromised manner, where the technician has noted his/her uncertainty, increases the incidence of NRNP cases. This may suggest that cows likely to be NRNP have an observable abnormality at the time of first insemination.

The variation between the sire of the semen used and the incidence of NRNP cases relative to those non-pregnant cows returning for a second service with in 24 days has not been reported previously. Its effect may be on the timing of embryo mortality, with those sires associated with reduced or more immediate embryo mortality resulting in an increased potential of the cow to return to oestrus within 24 days as compared with other sires that may be associated with an increased or more delayed embryo mortality.

In conclusion, the 18% incidence of non-pregnant cows which do not return for a second service within 24 days demonstrates the commercial importance of this form of sub-fertility. The six variables consistent in both final models were the interval from calving to MSD, protein composition of milk in the first 120 days of lactation, age, retained foetal membranes, diagnosis and treatment with synchrony drugs, and herd. Other variables included in only one final model were the certainty of insemination, vaginal discharge, carrying over non-pregnant cows, and the sire of the semen used. This study has identified possible at-risk groups suitable for early veterinary intervention.

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**Table 1**. Reasons for excluding cases from the screened data set.

| Reason for exclusion | Number of cases |
|---|---|
| Cows with missing data or atypical breeding programs. Cases include cow that:<br>- were served before mating start date<br>- died, were culled or "to be culled" before mating start date<br>- died during mating<br>- were embryo transfer donors or recipients<br>- were "to be culled" during mating<br>- had no final pregnancy test | 2549 |
| Herds with less than a 25 day artificial breeding period | 1503 |
| Cows which were not inseminated at the first heat during joining | 150 |
| Cows with a first service 23 days or less before the end of the AI period | 4894 |
| Cows with no heat or serve recorded during the artificial breeding period | 1240 |
| Herds reduced to insufficient cows for comparison | 36 |
| Cows with the second cycle date coded as a heat rather than a serve | 37 |
| *Total Exclusions* | *10409* |

**Table 2**. Definition of explanatory variables included in the analysis of the incidence of non-return, non-pregnant cows (NRNP), and the number of cases of each level of the variables.

| Definition of variables | Level | No. Cases (or range) |
|---|---|---|
| *Cow descriptions* | | |
| Herd [a,b] | 114 levels (herds) | 17 to 368 |
| Age [a,b] | 2 years old | 3020 |
| | 3 years old | 2601 |
| | 4 years old | 2265 |
| | 5 to 7 years old | 4108 |
| | 8 years and older | 2008 |
| Breed [b] | Holstein-Friesian | 9891 |
| | Jersey | 1424 |
| | H-F Crossbred | 1759 |
| | Other | 658 |
| Interval from calving to the MSD [a,b c] | continuous from -59 to 130 days | |
| *Variables to describe the last calving record* | | |
| Carried over [a,b] | No | 13050 |
| | Carried over one year | 332 |
| | Carried over two years | 402 |
| Aborted [b] | No | 14369 |
| | Yes | 55 |
| Induced to calve [a] | No | 12945 |
| | Yes | 1479 |
| Twin calves [a] | No | 14258 |
| | Yes | 166 |
| Retained foetal membranes [a,b] | No | 13828 |
| | Yes | 596 |
| Vaginal discharge [a,b] | No | 14354 |
| | Yes | 70 |
| Dystocia [b] | No | 13497 |
| | Yes | 927 |

| | | |
|---|---|---|
| *Variables to describe the first insemination* | | |
| Technician certainty of insemination Success [a] | Yes | 13690 |
| | Doubtful | 427 |
| Sire used (semen) [a,b] | 30 bulls of frequent use | 94 to 946 |
| | other bulls | 7034 |
| Semen storage [a] | Off farm | 6333 |
| | On farm | 7764 |
| Semen straw split for multiple Inseminations [a,b] | No | 12780 |
| | Yes | 1367 |
| State of storage of semen [b] | Frozen | 13770 |
| | Fresh | 377 |
| Time of insemination [b] | am | 9106 |
| | pm | 4907 |
| Qualification of inseminator | Professional | 10194 |
| | Do-it-yourself | 3669 |
| Pre-service diagnosis and treatment [a,b d] | No synchrony drugs | 12216 |
| | PG synchrony drugs | 572 |
| | Treated anoestrous cows | 705 |
| *Variables to describe milk production* | | |
| Milk yield in 120 days of lactation | 2000L or less | 1601 |
| | 2001 to 3000L | 6813 |
| | 3001 to 4000L | 4876 |
| | 4001L and greater | 982 |
| Milk protein (as a % of volume) for 120 days of lactation [a,b] | continuous from 2.5 to 4.9% | |
| Milk protein (as a yield) for 120 days of lactation [a,b] | continuous from 16 to 209kg | |
| Milk fat (as a % of volume) for 120 days of lactation [b] | continuous from 1.8 to 7.5% | |
| Milk fat (as a yield) for 120 days of lactation [a,b] | continuous from 13 to 303 kg | |
| *Variables to describe genetic merit* | | |
| Sire ABV [e] for Milk yield [b] | 500 or less | 1687 |
| | 500 - 1000 | 3609 |
| | 1000 - 1500 | 3775 |
| | Higher than 1500 | 1093 |

| | | |
|---|---|---|
| Sire ABV [e] for Fat yield | 30 or less | 3878 |
| | 30 - 40 | 2394 |
| | 40 - 50 | 3197 |
| | Higher than 50 | 695 |
| Sire ABV [e] for Fat composition (% of volume) [a] | -0.25% or less | 3189 |
| | -0.25% - 0.00% | 3348 |
| | 0.00% - 0.25% | 2460 |
| | Higher than 0.25% | 1167 |
| Sire ABV [e] for Protein yield | 20 or less | 2969 |
| | 20 - 30 | 4192 |
| | 30 - 40 | 2100 |
| | Higher than 40 | 903 |
| Sire ABV [e] for Protein composition (% of volume) | -0.2% or less | 1711 |
| | -0.2% to -0.1% | 3990 |
| | -0.1% to 0.0% | 2842 |
| | Higher than 0.0% | 1621 |

[a] Variable retained at bivariate screening step to be offered to the logistic model comparing NRNP cows to cows pregnant to the first service.
[b] Variable retained at bivariate screening step to be offered to the logistic model comparing NRNP cows to cows returning to oestrus18 t0 24 days after first service (Return24).
[c] Mating Start Date. This is the first day of the defined period of the year when the herd owner aims to get all non-pregnant cows in calf.
[d] Synchrony treatment included prostaglandin (PG) or a progesterone based treatment for cows diagnosed as anoestrus (Treated anoestrous cows).
[e] ABV: Australian Breeding Value.

**Table 3**. Variables and variable categories in the final logistic regression models. The first model compared the incidence of non-return, non-pregnant cows with pregnant cows (NRNP:Pregnant). The second model compared the incidence of non-return, non-pregnant cows with those that returned to service (NRNP:Return24). Herd and sire used are not presented as they have too many categories.

| Variables | Logistic-regression model for NRNP: Pregnant [c] | | Logistic-regression model for NRNP: Return24 [d] | |
|---|---|---|---|---|
| | OR | 95% CI | OR | 95% CI |
| *Interval from calving to MSD* [a] | | | | |
| For every week increase | 0.86 | 0.84, 0.88 | 0.96 | 0.94, 0.98 |
| *Average Protein composition over 120 days* [b] | | | | |
| For every 0.1% increase | 0.95 | 0.93, 0.98 | 0.96 | 0.93, 0.98 |
| *Retained Foetal Membranes* | | | | |
| Not Observed | 1.00 | - | 1.00 | - |
| Observed | 2.65 | 2.08, 3.38 | 1.69 | 1.32, 2.17 |
| *Age at calving* | | | | |
| 2 years old | 1.00 | - | 1.00 | - |
| 3 years old | 0.84 | 0.72, 0.99 | 0.91 | 0.76, 1.10 |
| 4 years old | 0.78 | 0.66, 0.92 | 0.80 | 0.66, 0.97 |
| 5 to 7 years old | 0.82 | 0.70, 0.95 | 0.78 | 0.66, 0.92 |
| Older than 7 years | 1.15 | 0.96, 1.37 | 0.95 | 0.78, 1.16 |
| *Pre-service diagnosis and treatment* | | | | |
| No synchrony drugs | 1.00 | - | 1.00 | - |
| PG synchrony drugs | 1.08 | 0.82, 1.44 | 0.91 | 0.68, 1.21 |
| Treated anoestrous cows | 2.00 | 1.59, 2.54 | 1.49 | 1.17, 1.91 |
| *Certainty of insemination* | | | | |
| Yes | 1.00 | - | | |
| No | 2.54 | 1.81, 3.55 | | |
| *Vaginal discharge* | | | | |
| Not observed | 1.00 | - | | |
| Observed | 2.75 | 1.43, 5.28 | | |

| | | |
|---|---|---|
| *Was not pregnant during a previous seasonal breeding period* | | |
| No non-pregnant period | 1.00 | - |
| A non-pregnant period at least 12 months previous | 0.90 | 0.64, 1.26 |
| A non-pregnancy during the last season | 1.67 | 1.26, 2.23 |

[a] Mating Start Date. This is the first day of the defined period of the year when the herd owner aims to get all non-pregnant cows in calf.
[b] Protein composition is the proportion of protein measured in the cumulative 120 day milk yield.
[c] model deviance = 3722.0; model df = 123 ($p<0.001$); Hosmer and Lemeshow Goodness-of-Fit test $p=0.10$
[d] model deviance = 354.0; model df = 148 ($p<0.001$); Hosmer and Lemeshow Goodness-of-Fit test $p=0.67$

**Figure 1**. Odds ratios and 95% confidence intervals for 114 herds in the model determining the incidence of non-return, non-pregnant (NRNP) cows as compared with those cows that were pregnant to the first service. The reference herd had an average incidence of NRNP cows and over n=200 cases.

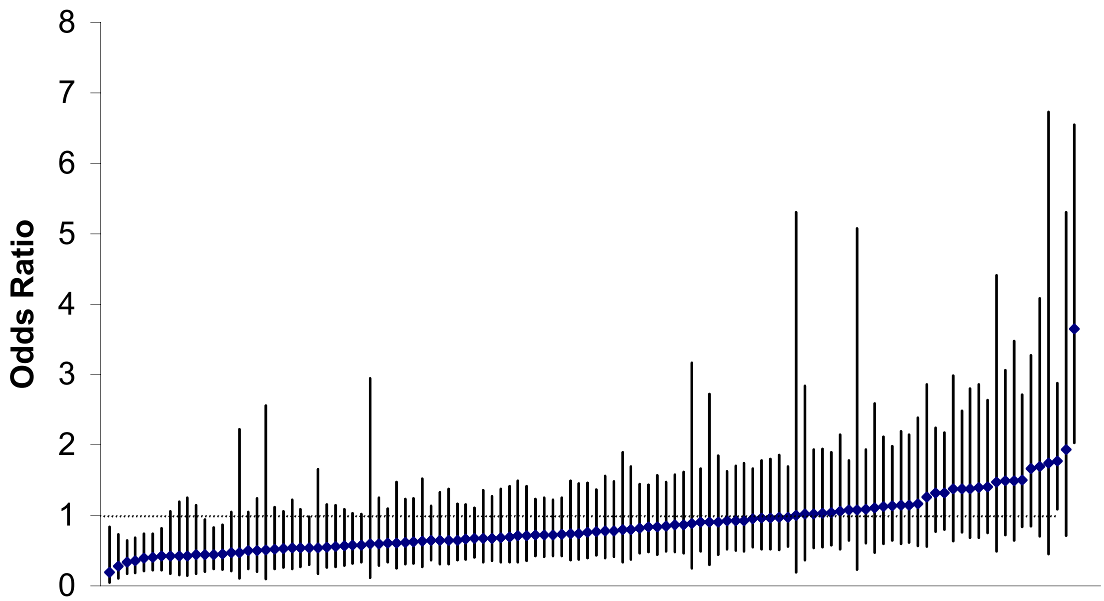
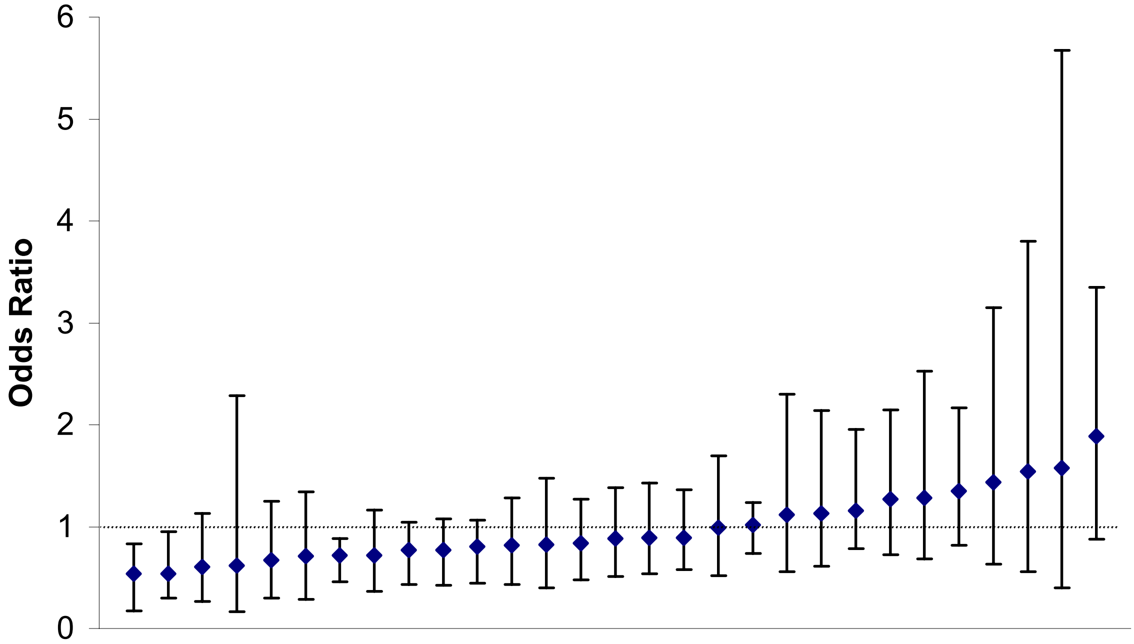


Figure 2. Odds ratios and 95% confidence intervals for 30 sires of semen used in the model determining the incidence of non-return, non-pregnant (NRNP) cows as compared with those cows that were not pregnant to the first service but did return for a second service within 24 days. The reference sire of semen group was a pooled group of miscellaneous sires.



## *Appendix 4: Epidemiological tables for analysing risk factors in the Maffra field experiment*

Abbreviations:

| | | | |
|---|---|---|---|
| BCS | Body Condition Score | Lower CI | Lower 95% Confidence Interval |
| NVO | No Visible Oestrus | Upper CI | Upper 95% Confidence Interval |
| OR | Odds Ratio | | |

**Analysis 1**: Risk factors associated with incidence of anoestrus (non-cycler or NVO) cows. Includes BCS at **calving** in the model.

Factors in model:
- Herd
- Breed
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at calving

### Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Herd | 1 | 198 | 1.00 | - | - |
| | 2 | 398 | 1.00 | 0.65 | 1.56 |
| | 3 | 395 | 0.71 | 0.46 | 1.09 |
| | 4 | 376 | 0.42 | 0.27 | 0.65 |
| Age | 2 | 263 | 1.00 | - | - |
| | 3 | 222 | 0.85 | 0.54 | 1.33 |
| | 4-6 | 501 | 0.59 | 0.40 | 0.87 |
| | 7+ | 381 | 0.71 | 0.48 | 1.06 |
| Calving interval | | | | | |
| For every week increase | | 1357 | 0.70 | 0.66 | 0.75 |
| Protein composition | | | | | |
| For every 0.1% increase | | 1361 | 0.82 | 0.76 | 0.88 |
| BCS at calving | | | | | |
| | 4.5 and less | 321 | 1.00 | - | - |
| | 4.75 to 5.25 | 584 | 0.87 | 0.62 | 1.22 |
| | >5.5 | 387 | 0.52 | 0.34 | 0.80 |

**Analysis 2**: Risk factors associated with incidence of anoestrus (non-cycler or NVO) cows. Includes BCS at a **pre-mating** visit in the model.

Factors in model:

- Herd
- Breed
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at pre-mating visit

## Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Herd | 1 | 198 | 1.00 | - | - |
| | 2 | 398 | 1.06 | 0.70 | 1.58 |
| | 3 | 395 | 0.61 | 0.41 | 0.92 |
| | 4 | 376 | 0.49 | 0.32 | 0.74 |
| Calving interval | | | | | |
| For every week increase | | 1357 | 0.76 | 0.72 | 0.80 |
| Protein composition | | | | | |
| For every 0.1% increase | | 1361 | 0.83 | 0.77 | 0.89 |
| BCS at premating visit | | | | | |
| | 4.5 and less | 297 | 1.00 | - | - |
| | 4.75 to 5.25 | 678 | 0.80 | 0.60 | 1.07 |
| | >5.5 | 231 | 0.47 | 0.31 | 0.71 |

**Analysis 3**: Risk factors associated with incidence of pregnant cows to first service.

Factors in model:

- Herd
- Breed
- Pre-mating treatment
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at calving

## Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Herd | 1 | 171 | 1.00 | - | - |
| | 2 | 379 | 1.27 | 0.85 | 1.90 |
| | 3 | 361 | 1.50 | 1.02 | 2.19 |
| | 4 | 321 | 0.99 | 0.67 | 1.48 |
| Calving interval | | | | | |
| For every week increase | | 1228 | 1.14 | 1.08 | 1.21 |
| Protein composition | | | | | |
| For every 0.1% increase | | 1227 | 1.11 | 1.04 | 1.18 |

**Analysis 4**: Risk factors associated with incidence of non-pregnant cows to first service returning within 24 days.

Factors in model:

- Herd
- Breed
- Pre-mating state (and treatment)
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at calving

## Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Herd | 1 | 171 | 1.00 | - | - |
| | 2 | 379 | 1.11 | 0.76 | 1.61 |
| | 3 | 361 | 0.69 | 0.47 | 1.02 |
| | 4 | 321 | 1.36 | 0.92 | 2.00 |

**Analysis 5**: Risk factors associated with incidence of phantom cows.

Factors in model:
- Herd
- Breed
- Pre-mating state (and treatment)
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at calving

## Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Treatment | No Trt | 363 | 1.00 | - | - |
| | PG | 424 | 1.32 | 0.88 | 1.97 |
| | CIDR | 407 | 2.45 | 1.66 | 3.62 |
| | CL-NVO | 38 | 2.25 | 0.98 | 5.16 |
| Calving interval | | | | | |
| For every week increase | | 1228 | 0.90 | 0.84 | 0.97 |

**Analysis 6**: Risk factors associated with incidence of cows which lost a pregnancy between an early pregnancy diagnosis and the 13-week examination. Includes BCS at **calving** in the model.

Factors in model:
- Herd
- Breed
- Pre-mating state (and treatment)
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at calving

## Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Herd | 1 | 171 | 1.00 | - | - |
| | 2 | 379 | 0.54 | 0.18 | 1.63 |
| | 3 | 361 | 0.21 | 0.07 | 0.64 |
| | 4 | 321 | 0.43 | 0.14 | 1.37 |
| BCS at calving | | | | | |
| | 4.5 and less | 295 | 1.00 | - | - |
| | 4.75 to 5.25 | 524 | 1.28 | 0.37 | 4.44 |
| | >5.5 | 352 | 3.65 | 1.01 | 13.20 |

**Analysis 6**: Risk factors associated with incidence of cows which lost a pregnancy between an early pregnancy diagnosis and the 13-week examination. Includes BCS at a **pre-mating** visit in the model.

Factors in model:
- Herd
- Breed
- Pre-mating state (and treatment)
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at pre-mating visit

## Significant factors

No significant factors

**Analysis 7**: Risk factors associated with incidence of cows which had an extended period of sub-luteal progesterone concentrations that indicated a period of post-insemination anoestrus or an extended follicular phase (Anoestrus).

Factors in model:

- Herd
- Breed
- Pre-mating state (and treatment)
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at calving

## Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Herd | 1 | 163 | 1.00 | - | - |
| | 2 | 361 | 0.74 | 0.25 | 2.20 |
| | 3 | 343 | 3.34 | 1.09 | 10.19 |
| Treatment | No Trt | 342 | 1.00 | - | - |
| | PG | 388 | 0.13 | 0.02 | 1.09 |
| | CIDR | 363 | 3.30 | 1.27 | 8.58 |
| Calving interval | | | | | |
| For every week increase | | 867 | 0.85 | 0.72 | 0.99 |
| Protein composition | | | | | |
| For every 0.1% increase | | 867 | 0.70 | 0.56 | 0.88 |

**Analysis 9**: Risk factors associated with incidence of cows which had an inter-oestrus interval greater than 24 days that **were detected** on heat at the second oestrus

Factors in model:

- Herd
- Breed
- Pre-mating state (and treatment)
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at calving

## Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Herd | 1 | 163 | 1.00 | - | - |
| | 2 | 361 | 0.38 | 0.17 | 0.86 |
| | 3 | 343 | 1.07 | 0.52 | 2.22 |
| Calving interval | | | | | |
| For every week increase | | 867 | 0.88 | 0.78 | 0.99 |
| Protein composition | | | | | |
| For every 0.1% increase | | 867 | 0.84 | 0.72 | 0.98 |

**Analysis 10**: Risk factors associated with incidence of cows which had an inter-oestrus interval greater than 24 days that **were not detected** on heat at the second oestrus

Factors in model:

- Herd
- Breed
- Pre-mating state (and treatment)
- Age
- Calving Interval
- Protein composition of milk from over 120 days
- BCS at calving

## Significant factors

| | | n | OR | Lower CI | Upper CI |
|---|---|---|---|---|---|
| Herd | 1 | 163 | 1.00 | - | - |
| | 2 | 361 | 1.76 | 0.79 | 4.42 |
| | 3 | 343 | 0.76 | 0.27 | 2.17 |