
VOLUME 62 2002

ISSN 0370-2731/2001

Proceedings of the New Zealand Society of Animal Production **61:** 180-183

Factors associated with the incidence of 'Phantom cows' in Australian dairy herds

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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 in which 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; DRDC, 2000) has identified that a proportion of cows that were retrospectively diagnosed 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, in which cows are synchronised for 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).

Preliminary analysis of the InCalf data (DRDC, 2000) has determined that 18% (2560/14,424) of cows inseminated early in the artificial breeding period exhibited this Phantom Cow syndrome (J. Morton & D. Nation, unpublished data). 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 which returned to oestrus 18 to 24 days later (n=3804) had 65% conception to artificial insemination and 26% to natural mating while 9% were not pregnant.

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

MATERIALS AND METHODS

Animals

Four commercial herds were involved in the study. All cows in Herd 1 were enrolled (n=196), while cows from Herds 2 to 4 were randomly selected (balanced for age and calving to mating start date interval within herd; 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 their 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.

The herd managers recorded oestrus events in the 30 days before the MSD and insemination dates for at least the first 42 days of mating. Cows that were not detected in oestrus by 8 days before the MSD were examined per rectum. Those diagnosed as anoestrus on the basis of no palpable corpora lutea 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 the 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). These cows were allocated to the earliest possible PG treatment when 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.

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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 between 28 and 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 further categorised into one of the following groups: 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).

The following explanatory variables were used to categorise the data: 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 (≤ 4.5, 4.75 to 5.25, or ≥ 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 have been reported.

RESULTS

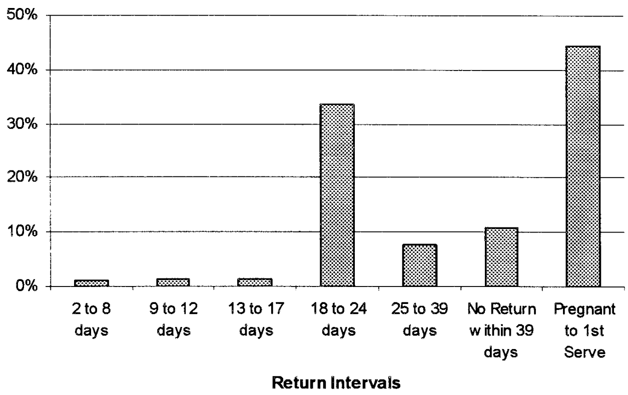
Animals

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.

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

	Herd				
Treatment	**1**	**2**	**3**	**4**	**Total**
An-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

FIGURE 1: The proportion of cows in each return to service interval for the study population. Cows that had not returned to service within 39 days were categorised as either pregnant (Pregnant to 1[st] Serve) or as having no return to service (No Return within 39 days).



Incidence of pregnant and Phantom cows

There were 19% of cows classified as Phantom cows, while 45% were pregnant to the first serve and 37% returned for a second service within 24 days of the first service (Table 2). There was a significant association between Phantom cows and physiological state before the first service, but not with herd, age, calving to MSD interval, and body condition score at calving. In contrast, both herd and physiological state before the first service were associated with pregnancy to the first service.

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 (28% vs. 16% vs. 13%; $P<0.05$).

Subcategories of Phantom cows

There were 89% of the Phantom cow cases subcategorised as Long Return, No Return or Anoestrus (Table 3). The proportion of Phantom cows classified as Long Return was associated with herd and also physiological state before the first service ($P<0.05$) with a higher incidence among untreated cows and treated anoestrous cows compared with PG treated cows. No Return cows had a higher incidence among PG-treated cows and were lowest among treated anoestrous cows. Cows four years and older had a reduced incidence of being anoestrus after first insemination, and those cows treated as anoestrus before the first insemination were more likely to have a continued (or recurring) anoestrus state after insemination ($P<0.05$). There was an increased tendency for cows calving with a BCS ≤ 4.5 to be Anoestrus ($P<0.10$; Table 3).

Diagnoses of Pregnancy

The diagnosis of pregnancy was accurate after excluding the gross abnormal cases of ovarian cysts, pyometra and adhesions. All pregnant cases (n=321) had a uterine lumen at least 15mm in diameter and an embryo ≥5mm in length.

TABLE 2: Relationship between various explanatory variables and the incidence (percent of total) of cows that conceived to first service (Pregnant Cows), returned to oestrus within 24 days of service (Returned to Service) or non-return, non-pregnant cows (Phantom cows). Those associations that were significant in the final multivariate model are marked with an * (P<0.05).

	n	Pregnant Cows	Returned to Service	Phantom Cows
Explanatory variable	721	45%	37%	19%
Herd				
Herd 1	169	45% *	34%	21%
Herd 2	208	38% *	43%	20%
Herd 3	180	61% *	22%	18%
Herd 4	164	35% *	48%	17%
Age				
2yo	130	45%	41%	15%
3yo	109	50%	31%	18%
4-6yo	279	46%	35%	19%
7+yo	203	39%	39%	22%
Calving to mating start date interval				
4 to 6 weeks	61	33%	39%	28%
7 to 9 weeks	198	40%	35%	25%
10 to 12 weeks	379	47%	37%	15%
13+ weeks	81	52%	35%	14%
Body Condition Score at calving				
≤4.5	178	38%	43%	19%
4.75 to 5.25	317	43%	38%	19%
≥5.5	211	53%	30%	17%
Treatment according to physiological state before first service				
An-CIDR Treatment	230	33% *	40%	28% *
PG Treatment	280	50% *	34%	16% *
No Treatment	211	50% *	37%	13% *

TABLE 3: Relationship between various explanatory variables and the incidence (percent of total) of Phantom cows that have ultrasonographic evidence of a CL on either ovary but did not return to service (No Return), cows that returned to service 25 to 39 days after insemination (Long Return), or cows with no CL on either ovary and did not return to service (Anoestrus). Those associations that were significant in the final multivariate model are marked with an * (P<0.05).

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

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

There were only 11 cases (3.3%) that appeared to have aborted. 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 should be of major concern to the Australian dairy industry that 19% of the study population were not confirmed pregnant to the first insemination and did not return for a second service within 24 days. An understanding of the factors associated with this phenomenon is crucial to improving the reproductive performance of dairy cows. This is the first report to investigate associations of the Phantom cow syndrome with age, interval from calving to MSD, BCS at calving, and intervention according to the physiological state before the first service.

It is likely that a major cause of the Phantom cow syndrome is due to 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 study was not able to differentiate between cows that were not detected in oestrus and those that were pregnant at this time and subsequently aborted. It is of note that the proportion of Phantom cows which were classified as No Return was lower amongst the CIDR-treated anoestrous cows, indicating that factors other than failure to detect oestrus were more prevalent for this group.

The cows that had a return to service interval between 25 and 39 days (Long Return) may have abnormal luteal function resulting in a prolonged oestrous cycle. 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. Long return intervals after a period of post-partum anoestrus have been observed previously (Ball and McEwen, 1998), but the difference between PG-treated cows and untreated cows has not been 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 regular oestrous cycles. It is possible that ovulation did not occur after the initial anoestrous CIDR 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 *et al.*, 1993).

The diagnosis of anoestrus after first insemination in a small number of cows in the PG and No Treatment groups indicated that there may still be a proportion of these populations that had resumed an anoestrous state after being observed in oestrus. Alternatively, they may have been chronically anoestrus and had a first insemination to a falsely detected oestrus (Rhodes *et al.* 1999). This hypothesis is unlikely as, in addition to the detection of oestrus at the time of insemination, these cows must have been observed in oestrus in the pre-mating period to be excluded from the anoestrous treatment.

In conclusion, Phantom cows are common among commercial dairy herds and are primarily made up of cows exhibiting a delayed return to service, with non-returning (possible caused by embryo mortality or failure to detect oestrus) and anoestrous cows also contributing significantly to this phenomenon. Treatment with prostaglandin before the first insemination reduced the incidence of delayed return to service, but this treatment was associated with an increased proportion of non-returning cows. 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

The authors would like to acknowledge the assistance from the herd owners and veterinarians, as well as technical staff. Genetics Australia generously provided pharmaceuticals for use in this experiment. This work was funded by a grant from the Dairy Herd Improvement Fund, Victoria, Australia.

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