Embryo mortality and early post-oestrous cycle embryonic death estimated from oestrous cycle lengths and milk progesterone analysis

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An estimate of embryo mortality (cycles longer than 28 days) was obtained from milk progesterone analysis and delayed return rate in two dairy herds. Oestrus cycle lengths were measured, and cycles grouped according to whether artificial insemination (AI) had, or had not, been performed. Early post-oestrous cycle embryonic death (cycles extended beyond the average of 21 days, yet less than 28 days in length) was estimated by examining the changes in distribution of the two groups of oestrous cycle lengths. Embryo mortality occurring between 28 and 75 days after AI was measured at 15,2% using progesterone analysis and 21,6% using delayed returns. A 23,3% increase ($P < 0{,}001$) in the number of extended cycles (22 – 28 days) in the mated group of cows was found. This change in distribution of the length of mated and unmated groups of cycles was significantly different, and was assumed to represent early post-oestrous cycle embryonic death. Some implications of these findings are discussed.

Embriomortaliteit (siklusse langer as 28 dae) is geskat vanuit melkprogesteroonbepalings en vertraagde terugkeertempo in twee melkkuddes. Siklusse is ingedeel in twee groepe volgens die voorkoms al dan nie van kunsmatige inseminasie (KI) en die sikluslengtes is gemeet. Vroeë embrio-afsterwing (siklusse langer as die gemiddeld van 21 dae, maar korter as 28 dae) is geskat deur die voorkoms van 'n verspreidingsverandering in estruslengte tussen die twee groepe. In die geïnsemineerde groep is 'n toename van 23,3% ($P < 0{,}001$) in die aantal verlengde siklusse (22 – 28 dae) gevind, terwyl embriomortaliteit tussen 28 en 75 dae na inseminasie bepaal is as 15,2% en 21,6% met behulp van melkprogesteroonbepalings en terugkeertempo onderskeidelik. Die verandering in die verspreiding van sikluslengte tussen geïnsemineerde en nie-geïnsemineerde groepe is betekenisvol verskillend, en word beskou as verteenwoordigend van vroeë embriomortaliteit. Enkele implikasies hiervan word kortliks bespreek.

Keywords: Embryo mortality, dairy cows, oestrous cycle lengths, milk progesterone analysis

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Introduction

It is widely considered that, in dairy cows, an increase in the interval between service and return to oestrus beyond the usual range of 17 – 25 days reflects embryo mortality (Erb & Holtz, 1958). Estimates of such losses have been based on the decline in non-return rate, slaughter experiments, and recently, analysis of milk progesterone content. In artificially inseminated herds these estimates have ranged from 7,2% (Kummerfeld, Oltenacu & Foote, 1978) to 24,7% (Bulman & Lamming, 1979).

The major portion of embryonic losses occurs well before day 15 after service (Ayalon, Weis & Lewis, 1968; Boyd, Bacsich, Young & McCracken, 1969). Approximately 40% of inseminations are followed by reproductive failure during this time (Pope & Swinburne, 1980). Neither the delayed returns nor the milk progesterone method is capable of detecting these early events. Cows experiencing such early death of the embryo will return to oestrus after the same interval as unmated animals. However, the length of the oestrous cycle may be extended slightly. Some early death of the embryo may therefore be represented by extension of the cycle to more than the average cycle length of unmated cows during the same post-partum period (Kummerfeld, *et al.*, 1978).

The aim of this analysis was to determine the extent of embryo mortality and early post-oestrous cycle embryonic death in two dairy herds where samples were being obtained for milk progesterone analysis. From the results it was possible to evaluate the importance of embryo mortality in commercial dairying.

Procedure

Whole milk samples (afternoon milking) were collected daily from 44 cows in two herds located on two research farms in the Natal Midlands. Cows were initially chosen for study on the basis of a poor reproductive history (long inter-calving periods and more than two inseminations) although all cows showed clinically normal reproductive tracts at the start of the study. However, when it appeared that previous reproductive history had little influence on the following reproductive period (Butterfield, 1986), cows were chosen for the study at random. Sampling was started between 10 and 20 days after calving and continued until pregnancy was confirmed by rectal palpation of the reproductive organs 60 days after service. Sampling was resumed if a cow returned to service after pregnancy had been confirmed. The routine for detection of oestrus in both herds consisted of twice daily observation prior to milking supplemented by casual observation of the herds during daylight hours.

The milk samples were analysed for progesterone content using the radioimmunoassay method as described by Butterfield (1986). A progesterone profile was plotted for each of the 44 cows under observation. The data obtained from these profiles were compared with observations for oestrus and insemination dates. Oestrous cycles which occurred before 30 days post-partum were excluded from the analysis. The lengths of 150 oestrous cycles were determined from progesterone profiles and oestrous observations and categorized into either mated (AI performed at oestrus) or unmated (no AI performed at oestrus) cycle groups.

Where expected heat was not observed, the end of the previous cycle was taken to have occurred at the mid-point of the trough where progesterone levels were less than 1 ng/ml milk. In fact, 33 out of a possible 150 heats were missed. The estimate of embryo mortality in the cow cycles was based on a sustained production of progesterone during the first 28 days after breeding, followed by a sudden decline in progesterone levels, with a return to cyclic progesterone patterns during the 28 to 75-day interval. This pattern was assumed to indicate that pregnancy had been initiated and terminated.

Embryo mortality was also estimated using the expected date of return to oestrus following insemination as basis, i.e. the delayed returns method. The decline in non-return rate during the interval between 28 and 75 days then estimated the proportion of cows in which the embryo died (Kummerfeld, *et al.*, 1978). This analysis was performed to determine the inaccuracy of the delayed returns method with respect to unobserved oestrous periods.

The incidence of early embryonic death was calculated after determination of the average length of an oestrous cycle in an unmated cow. Any increase in frequency of long cycles among inseminated cows could thus be assumed to represent early embryonic death. The difference in distribution of cycle lengths for mated and unmated oestrous cycles was thus analysed.

Results

An estimate of embryo mortality (cycles longer than 28 days) of 15,2% was calculated from the milk progesterone profiles. Of this, 58% occurred during the period between 30 and 38 days after insemination. This indicated that embryos enter a critical survival stage during this time.

Using the delayed returns method of estimating embryo mortality it was found that 21,6% of the cow cycles represented delayed returns to oestrus, which was indicative of embryo loss. The discrepancy between this result and that calculated from milk progesterone profiles was due to unobserved oestrous periods.

The estimate of early embryonic death was based on the calculation of the mean length of oestrous cycles of cows which had not been inseminated at oestrus. The value obtained from this calculation was 21,02 days. Using this value the mated and unmated oestrous cycles were distributed according to length of cycle and the difference in distribution calculated (Table 1).

Table 1 Length and distribution of oestrous cycles in mated and unmated dairy cows

Days after previous oestrus	Mated		Unmated		Differences
	n	%	*n*	%	%
0 – 21	23	29,1	48	67,6	–38,5
22 – 28	44	55,7	23	32,0	+23,2
0 – 28	67	84,8	71	100,0	–15,2
> 28	12	15,2	0	0	+15,2
Total	79	100,0	71	100,0	

A marked change in the distribution of cycle lengths before and after insemination was evident (Table 1). The inseminated group had 38,5% fewer cycles of 21 days of less, 23,3% more cycles 22 – 28 days long and 15,2% more cycles longer than 28 days. According to the X^2 test, these differences were significant ($P < 0,001$). It was expected that, as a result of embryo mortality, the length of some oestrous cycles would be extended after AI. In fact, after insemination 70,9% of oestrous cycles were longer than 21 days compared to 32,4% such cycles in uninseminated cows ($P < 0,01$). Insemination of dairy cows thus causes a change in the distribution of oestrous cycle lengths.

Further evidence of this change is shown by the shift in distribution of cycle lengths of inseminated cows (Figure 1). This explains why the average cycle length of the mated group of cows was 25,54 days compared to 21,01 days for the unmated oestrous cycles. However, cycles longer than 28 days added considerable bias to the calculations. Exclusion of these long cycles from the analysis resulted in a mean cycle length of 22,35 days for the mated group. The 1,34 days difference between the means did not seem to warrant further analysis.

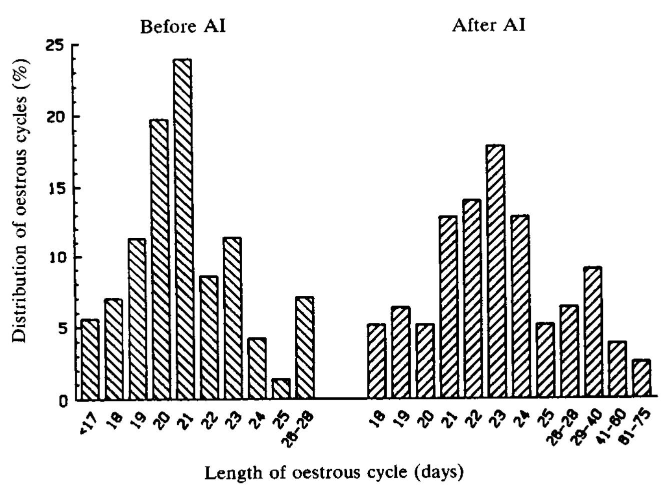

Figure 1 Distribution of oestrous cycle lengths before and after AI

However, as the standard deviation of the mean of both groups was similar (2,30 vs 2,29). the difference in variance about the means was compared. The difference was significant at the 1% level (t = 3,22, 136 df).

These results demonstrated that when dairy cows had the chance to become pregnant, but appeared not to conceive, the length of oestrous cycles was increased. The implication of this finding is important to dairy herd reproductive management.

Discussion

The analysis of milk progesterone levels is probably the most accurate non-slaughter method of estimating embryo mortality. Bias may be introduced to the estimate in cases of prolonged luteal activity in the non-pregnant cow, but in the absence of any apparent clinical abnormality the incidence of this is very low, approximately 1,5% (Bulman & Lamming, 1977). The 15,2% incidence of embryo mortality occurring after 28 days compares with the 12,5% estimate of Claus, Karg, Zwiauer, von Butler, Pirchner & Rattenberger (1983) and the 17% estimate calculated by MacFarlane, Booth, Deas & Lowman (1977) using similar methods.

Using the delayed return rate, the estimate of embryo mortality was 21,6%. A similar level of 22,7% was found by Kummerfeld, *et al.* (1978) when using the same technique. The difference between the 15,2% estimate and the 21,6% estimate demonstrated that a portion of the estimate obtained using delayed returns was biased due to unobserved heats. In this study, the bias introduced was fairly low (6,4%) as a result of efficient heat detection programmes in the herds involved. On farms where the efficiency of oestrous observation is poor, estimates of embryo mortality could be considerably influenced by unobserved heats.

The most important finding of this research was that the oestrous cycles of inseminated cows were longer than those of uninseminated cows. Kummerfeld, *et al.* (1978) suggested that cycles extended beyond 21 days in inseminated cows were associated with early post-oestrous cycle embryonic death, but could show no statistical significance. However, Boyd (1973) found a significant difference between inter-oestrous intervals before and after first AI, with 90,5% of pre-insemination cycles of normal length (18 – 24 days) compared with 43,5% of post-insemination cycles. Although Boyd's study was based on the delayed returns method, and bias due to unobserved heats is possible, the results are still highly significant for the present investigation.

The results of this analysis demonstrate that there is a significant difference between the lengths of inseminated and non-inseminated cycles. As all the cycles occurred during the same post-partum period, the difference in length must be the result of AI. Early post-oestrous cycle embryonic death is thus incriminated.

In the field of reproductive management of dairy herds, emphasis has been placed on improving conception rates (Watson, 1982; Watson & McDonald, 1984). The factors influencing the conception rate of inseminated dairy cows have been investigated (Shanks, Freeman & Berger, 1979; Stevenson, Schmidt & Call, 1983), but it seems that the emphasis should be placed on improving embryo survival.

When death of the conceptus occurs sufficiently early, the length of the oestrus cycle may be extended slightly or not affected at all. As there is no subsequent visible infertility, the economic consequences are the same as failure of fertilization. In fact, a large proportion of the estimated fertilization failure could probably be ascribed to early embryonic death. Failure to conceive may be replaced by failure to maintain the conceptus.

Bishop (1964) contended that a considerable part of embryo mortality was unavoidable, should be regarded as normal, and was a means of eliminating unfit genotypes at low biological cost. However, the extent of the embryo mortality phenomenon measured in this study is reproductively inefficient, and as such would be unacceptable to the commercial dairy farmer. Factors affecting embryo survival should thus be investigated further.

The increase in the length of oestrous cycles of inseminated cows was also relevant to the milk progesterone pregnancy test. Conventionally, the sampling day for the test is 21 – 22 days after AI (Pennington, Spahr & Lodge, 1976; Pope, Majzlik, Ball & Leaver, 1976). The results of this paper indicated that sampling at a slightly later date, say 24 – 25 days after AI, might prove to be more accurate. Heap, Holdsworth, Gadsby, Laing & Walters (1976) and Butterfield (1986) found that maximum accuracy for pregnancy diagnosis was obtained from samples taken 24 days after AI. These results endorse the findings of this trial.

Conclusion

If the increase in frequency of long cycles (longer than 21 days) among inseminated cows is assumed to represent embryonic death, the incidence of this phenomenon totalled nearly 40% (23,2% and 15,2%) in this study. Poor dairy herd management could compound the problem considerably, resulting in drastic economic consequences. Further research is thus warranted.

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