Effect of artificial insemination on submission rates of lactating dairy cows synchronised and resynchronised with intravaginal progesterone releasing devices and oestradiol benzoate

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Received 23 August 2004; received in revised form 12 January 2005; accepted 12 January 2005
Available online 10 March 2005

Abstract

This study investigated the hypothesis that a reduction in submission rates at a resynchronised oestrus is not due to the resynchrony treatment involving intravaginal progesterone releasing devices (IVDs) and oestradiol benzoate (ODB) but is associated with artificial insemination (AI) at the first synchronised oestrus. In Experiment 1, cows were synchronised for first oestrus with IVDs, with ODB administered at the time of device insertion (Day 0, 2 mg IM) and 24 h after removal (Day 9, 1 mg IM) and PGF2α injected at the time of device removal. Cows were then either inseminated (I) for 4 days or not inseminated (NI) following detection of oestrus (first round of AI). Every animal was resynchronised for a second round of AI by reinsertion of IVDs on Day 23 with administration of ODB (1 mg IM) at the time of insertion as well as 24 h after removal (Day 32). Cows detected in oestrus and inseminated for 4 days at the second round of AI were resynchronised for a third round by repeating the resynchrony treatment starting on Day 46 and inseminating cows on detection of oestrus for 4 days. In Experiment 2 the same oestrous synchronisation and resynchronisation treatments were used, but the timing of treatments differed. The cows had their cycles either presynchronised (treatment start Day −23) without AI and then resynchronised, starting on Day 0, for the first round of AI for AI at detected oestrus for 4 days, or they were synchronised (treatment start Day 0) for the first round of AI.

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In Experiment 1, 91.4% (64/70) and 92.6% (63/68) \((P = 0.79)\) of cows in the I and NI treatments, respectively, were detected in oestrus after the initial synchronisation. At the second round of AI, submission rates for insemination were lower in the I group compared to the NI cows (74.5%， 35/47 versus 92.6%， 63/68, respectively; \(P = 0.007\)). Pregnancy rates (proportion treated that were classified as becoming pregnant) in I and NI cows 4 weeks (61.4%, 43/70 versus 63.2%, 43/68) and 7 weeks (77.1%, 54/70 versus 69.1%, 47/68) after the AI start date (AISD) did not differ significantly between treatments. In Experiment 2, presynchronisation and then resynchronisation of oestrous cycles before the first round of AI did not affect oestrous detection rates at the first round of AI (100%, 44/44 versus 98.0%, 50/51; \(P = 0.54\)), or pregnancy rates 1 week (63.6%, 28/44 versus 60.8%, 31/51; \(P = 0.70\)), 4 weeks (72.7%, 32/44 versus 76.5%, 39/51; \(P = 0.76\)) and 7 weeks (81.8%, 36/44 versus 88.2%, 45/51; \(P = 0.40\)) after AISD compared to cows that had their cycles synchronised for the first round of AI. These findings support our hypothesis that a reduction in submission rates at a resynchronised oestrus is associated with AI at the first synchronised oestrus and not due to a resynchrony treatment involving IVDs and ODB. This study supports the concept that early embryonic loss following AI at a synchronised oestrus could cause a reduction in submission rates following resynchronisation of oestrus, although investigation of the effect of passing an AI catheter or semen components were not studied per se.

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**Keywords:** Synchronisation of oestrus; Resynchronisation; Artificial insemination; Detection of oestrus (submission rate)

### 1. Introduction

Synchronisation of oestrus in cattle has been used to facilitate the use of artificial insemination (AI) and to minimise the time needed to detect oestrus in cattle (Stevenson et al., 1999). Treatments have been recently developed using intravaginal progesterone releasing devices (IVDs) combined with injected oestradiol benzoate (ODB) to enable oestrous cycles in cows inseminated at an initial synchronised oestrus to be resynchronised for a second and even a third oestrous cycle (Cavalieri et al., 2000; Cavalieri and Macmillan, 2002; McDougall and Loeffler, 2004). Synchronisation of three consecutive oestrous cycles gives cows three opportunities to conceive to AI within a 7-week period of time with most cows returning to oestrus within 4 days at each synchronised oestrus. Using these treatment programs in Australian dairy herds that are managed with a seasonal breeding and calving pattern has produced pregnancy rates averaging 72% (2659/3717) within 7 weeks of the first day of inseminating (AI start date (AISD); Cavalieri et al., 2000). Maximising the cumulative proportion of the herd that conceives within the first 6 weeks of the breeding season has been shown to have a significant impact on the reproductive performance of seasonal calving herds (Morton, 2000a).

Whereas the percentage of cows that are detected in oestrus at a synchronised oestrus usually exceeds 90%, the percentage of non-pregnant cows that are detected in oestrus and submitted for a second AI (submission rate) at a resynchronised oestrus is usually less, ranging from 66 to 81% (Cavalieri et al., 2000, 2003a; Cavalieri and Macmillan, 2002). Factors that may cause a reduction in submission rates at a resynchronised oestrus include a failure to detect oestrus, failure of the resynchrony treatment to successfully resynchronise
Results from a recent study have suggested that over 90% of cows that return to oestrus following resynchrony can be detected in oestrus (Cavalieri et al., 2003b). Most cows that were not detected in oestrus in that study were in the luteal phase of the oestrous cycle at the time of the resynchronised oestrus. Failure of cows to enter oestrus was cited as a major factor reducing submission rates at a resynchronised oestrus in that study rather than a failure to detect oestrus. What could not be determined was whether or not the treatment that was used to resynchronise oestrous cycles was extending the luteal phase of some oestrous cycles and reducing submission rates at the resynchronised oestrus. This and other studies (Van Cleeff et al., 1991) have also shown that early embryonic loss is likely to be an important factor reducing the submission rates of cows with resynchronised oestrous cycles and differences between pregnancy rates and non-return rates. Cows that are pregnant at the time of the resynchrony treatment but subsequently lose a pregnancy can have prolonged interoestrus intervals and may not enter oestrus at the time when oestrus in non-pregnant and resynchronised cows is expected.

The aim of this study was to investigate the role played by AI in reducing submission rates at a resynchronised oestrus following the use of IVDs and ODB to synchronise and resynchronise oestrus. Our hypothesis was that a reduction in submission rates at a resynchronised oestrus was not due to use of IVDs and ODB to synchronise and resynchronise oestrus. Our hypothesis was that a reduction in submission rates at a resynchronised oestrus was not due to use of IVDs and ODB to synchronise oestrous cycles but was associated with AI at the first synchronised oestrus.

2. Materials and methods

2.1. Experimental design and animals

The study consisted of two experiments. Experiment 1 was designed to test the effect of AI at first synchronised oestrus on submission rates and reproductive performance following the resynchronisation of oestrous cycles. In Experiment 2 oestrous detection rates were compared in cows that had been synchronised for first oestrus and not inseminated, and then had oestrous cycles resynchronised compared to cows that were treated to synchronise first oestrus. A schematic outline of the experimental treatments undertaken for the two experiments is given in Fig. 1.

Cows in Experiment 1 were sourced from three seasonally calving dairy herds and had oestrous cycles synchronised for first insemination and then resynchronised for two successive oestrous cycles. The mean (±S.E.M.) interval from calving to AISD interval for cows in this experiment was 51.1 ± 8.3 days. Cows were allocated to two treatment groups with groups in individual herds balanced for calving to AISD interval, body condition score (BCS) and age to reduce differences between treatments. Body condition scores were made on a 1–5 scale using the system previously described by Ferguson et al. (1994). The two treatment groups consisted of cows that were inseminated at the first synchronised oestrous cycle (inseminated group; I) and cows that were deliberately not inseminated at the first synchronised oestrous cycle (not inseminated group; NI).

Cows assigned to Experiment 2 had been calved 309.8 ± 8 days (mean ± S.E.M.) before the AISD. The cows had been part of a breeding program that had been conducted in the
Fig. 1. Diagrammatic outline of treatments for Experiments 1 and 2. In Experiment 1, cows had oestrous cycles synchronised for a first oestrus with intravaginal progesterone releasing devices (IVD, with oestradiol benzoate (ODB) administered at the time of device insertion (Day 0, 2 mg IM) and 24 h after removal (Day 9, 1 mg IM). Prostaglandin F$_2$-\alpha (PG) was administered at the time of device removal (Day 8). In Experiment 1 cows were either inseminated or not inseminated on detection of oestrus (first round of AI) for 4 days after removal of devices. To synchronise cows for a second round of AI, IVD’s were reinserted on Day 23 and ODB (1 mg IM) was administered at the time of insertion and 24 h after removal of devices (Day 32). Cows detected in oestrus at the second round of AI were resynchronised for a third round of AI by repeating the resynchrony treatment commencing on Day 46. Blood samples were collected from a subset of cows for progesterone assay on Days 34 and 45. In Experiment 2 the same oestrous synchronisation and resynchronisation treatments that were used Experiment 1 were applied. One group of cows was, however, presynchronised by starting the oestrous synchronisation treatment on Day 23 (resynchronised first round), while a second group of cows had the treatment start from Day 0 (synchronised first round). Cows were not inseminated before Day 8. A new device was used between Days 23 and 15 in the presynchronised cows, and Days 0 and 8 in both the presynchronised and synchronised cows in Experiment 2. Following the first round of AI cows had oestrous cycles resynchronised for a second and third round of AI as in Experiment 1. Blood samples were collected from a subset of cows for progesterone assay on Days 11 and 22.
preceding spring–summer season in Herd C, but were classified as not pregnant following the completion of that program. Two breeding programs are conducted each year as part of the routine reproductive management of cows in Herd C (a winter breeding program and a spring–summer breeding program). Cows that do not become pregnant following the first program following calving are rebred at the second program. Cows which fail to become pregnant after two successive programs are removed from the herd. This system of mating is colloquially known as a “split-calving” herd.

Cows in Experiment 2 were assigned by odd or even ear tag number to one of two treatment groups. The first consisted of a “presynchronisation” group that had oestrous cycles synchronised for a first oestrus but were not inseminated at that oestrus. The timing of this treatment was such that cows in the second treatment (“synchronisation” treatment) started the treatment (Day 0) to synchronise oestrous cycles the same day that cows in the presynchronisation group started treatment that was used to resynchronise oestrous cycles. Thus, cows in Experiment 2 were given the same oestrous synchronisation and resynchronisation treatment as cows in Experiment 1 with the exception that the timing of the start of the first treatments in the presynchrony group preceded treatment in the other cows by 23 days. Cows in each study were fed predominantly on pasture supplemented with cereal grain at the time of milking.

2.2. Oestrous synchronisation treatment

Cows in Experiment 1 and the synchronisation group of cows in Experiment 2 were treated with an IVD impregnated with 1.9 g of progesterone (CIDR-B, Genetics Australia, Bacchus Marsh, Vic.) and an IM injection of 2.0 mg of ODB (Cidirol, Genetics Australia, Bacchus Marsh, Vic.) concurrently with IVD application (Day 0). Inserts were removed 8 days later when 25 mg of prostaglandin F2α (Lutalyse Solution, Pharmacia Animal Health, Rydalmere, NSW) was injected IM. Cows were then administered 1.0 mg of ODB IM 24 h after IVD removal. This same treatment was applied to cows in the presynchrony group of Experiment 2; but treatment was started on Day 23. On Day 0, cows in the presynchrony group in Experiment 2 were treated with a new IVD device and injected with only 1.0 mg of ODB. A new device was used between Days 0 and 8 in the presynchronised and synchronised cows in Experiment 2 so that effects due to the type of IVD used (new or used) could be excluded from causing any differences arising between these treatments. An injection of prostaglandin F2α was not given to the presynchronised cows on Day 8 but they were injected with 1.0 mg of ODB IM on Day 9 (Fig. 1).

Treatments to synchronise oestrus in cows for a second and third round of AI were the same for all cows enrolled in Experiments 1 and 2. The IVDs used to synchronise cows for the first round of AI were washed, disinfected and reinserted on Day 23 of the program with 1.0 mg of ODB being administered IM concurrently. All of the cows that were treated on Day 0 were retreated on Day 23. Eight days later (Day 31), inserts were removed and 1.0 mg of ODB was administered IM 24 h later (Day 32). On Day 46 of the program, inserts that had been used to synchronise cows for the first two consecutive oestrous cycles were again washed, disinfected and reinserted into cows that had been inseminated between Days 31 and 35 and 1.0 mg of ODB was administered IM. Eight days later inserts were removed and 24 h later (Day 55) cows that were treated with an IVD between Days 46 and
54 were administered 1.0 mg of ODB IM. After each period of treatment with an IVD any cow detected in oestrus 24 h after removal of devices was not treated with OBD. This was because the purpose of the treatment with OBD after removal of IVDs is to induce and synchronise oestrus, and this was unnecessary in animals detected in oestrus when ODB was administered. The number of animals not treated with ODB after removal of devices at each round of AI in Experiments 1 and 2 was <2.0% of the cows treated with an IVD. Previous studies support the effectiveness of using and reusing IVDs of the type used in this study for synchronising oestrus in dairy cows over three consecutive oestrous cycles (Cavalieri et al., 2000).

2.3. Detection of oestrus

Oestrus was detected using tail chalk (Herd A), or tail-paint (Herds B and C) at each synchronised oestrous cycle. Heatmount detectors (Kamar Heatmount detectors, Agri-Gene, Wangaratta, Vic.) were also used at the second and third rounds of AI in addition to tail-paint in Herd B to aid in the detection of oestrus in that herd. Aids for the detection of oestrus were applied to cows on the day or the day before IVDs were removed following treatment to synchronise cows for a first, second or third round of AI (Days 8, 31 and 54). Cows were monitored for oestrus twice daily for 4 days from the time of removal of IVDs. The system described by Macmillan et al. (1988) was used to record changes in tail chalk and tail-paint. The system described by Cavalieri et al. (2003c) was used to describe changes in heatmount detectors.

2.4. Artificial insemination

Artificial insemination was carried out for 4 days following the removal of IVDs at the three consecutive rounds of AI. These periods of insemination were defined as the first, second and third rounds of AI, respectively. Cows in the NI group, in Experiment 1 were deliberately withheld from insemination at the first round of AI. Inseminations were carried out by professional technicians using semen processed in caprogen (Shannon and Vishwanath, 1995) at the first round of insemination in Herds A and B and thawed frozen semen in Herd C. Thawed frozen semen was used at the second and third rounds in each herd. Bulls were run continuously with herds between Days 36 and 54 (after the second round of AI), and 59 to at least Day 66 (after the third round of AI).

2.5. Pregnancy diagnosis

Pregnancy diagnosis was carried out 13 weeks after the first round of AI. Pregnancy diagnosis was undertaken by ultrasonography (Aloka SSD–500, 5.0 MHz transducer, Medtel Pty Ltd., Oakleigh, Vic.) with the transducer fixed to a flexible metal extender. Conception dates were determined from visual estimation of foetal size and insemination dates. Where the estimated gestation length did not match with insemination dates and coincided with a period of time when bulls were running with the herd, conception dates were estimated from foetal size as observed with ultrasonography (Kålhn, 1989). Cows that were not detected in oestrus within 4 days of removing IVDs at the third round of AI and conceived
to a bull after that date were classified as not becoming pregnant within 7 weeks of the AISD.

2.6. Blood sampling and progesterone assay

Blood samples were collected from a random selection of cows from each treatment group in both studies and assayed for concentrations of progesterone in plasma. Blood was collected from the coccygeal vein or artery into evacuated tubes containing lithium heparin as an anticoagulant on Days 34 and 45 of Experiment 1 and Days 11 and 22 of Experiment 2. Plasma was separated within 3 h of collection and then frozen until the time of assay. Concentrations of progesterone in samples were determined using a radioimmunoassay kit (Spectria Veterinary Progesterone RIA kit, Orion Diagnostica, Espoo, Finland). The sensitivity of the assay was 0.12 ng/mL. The inter- and intra-assay coefficients of variation for plasma pools of 0.54, 4.2 and 8.1 ng/mL were 13.0 and 24.0%, 8.7 and 19.2%, and 7.3 and 14.2%, respectively.

2.7. Statistical analysis

All analyses were performed using the statistical package SPSS 11.0 for Windows 2001 (SPSS Inc., Chicago, IL, USA). Due to the relatively small numbers of cows in each treatment within each herd (≤28) in Experiment 1, effects due to age, BCS and calving interval were not included in analyses of binomial data. Fischer’s test was used for binomial data where proportions in some groups were or approached 0 or 100%, or the number of cows in treatment groups was <20. This included a comparison of 1-week pregnancy rates in Experiment 1 and data summarised in Table 2. Effects of the interaction of herd with treatment were not examined in such cases but data for individual groups is provided so as variation between groups across herds could still be assessed. Other proportional responses between treatment groups were compared using logistic regression analysis.

The AISD to conception intervals were compared using analysis of covariance with treatment and BCS entered as factors in the model, herd was entered as a random effect and age and calving to AISD intervals entered as covariates. In the analyses conducted for this study, Herd was considered to be a random effect and while included in logistic regression and ANOVA models effects due to herd were not reported. The interaction of treatment with herd was included in initial models. Means of treatment groups tested by ANOVA are adjusted means, corrected for the other factors in the model. Each of the presented means is reported with its standard error.

Fisher’s test was used with data from Experiment 2 to compare oestrous detection rates at the first round of AI and submission and conception rates at the second round of AI. Numbers of cows classified as not-pregnant-not-detected-in-oestrus were considered too small (n = 5 per group) to enable reliable estimates of the effects of age and BCS and these were omitted from this analysis. Cows were classified as not-pregnant-not-detected-in-oestrus if they were classified as not pregnant to insemination at the first round of AI and were not detected in oestrus at the second round of AI, that is, within 25 days of the first AI.

Other proportional responses between treatments in Experiment 2 were compared using logistic regression analysis. Treatment (presynchronisation and synchronisation) was
kept as a factor in each logistic regression model, while the explanatory variables were considered for elimination from the model using step-wise logistic regression (Collett, 1991). The test for elimination was a likelihood-ratio test, using a level of significance of 0.10. Probability values for all main effects remaining in models were determined using the approximate chi-squared distribution of the likelihood ratio statistic. Odds ratios and 95% confidence intervals were also calculated for all main effects remaining in models. Explanatory variables included in the model in Experiment 2 were age and BCS (≤2.5, 2.75, ≥3.0). The interval from calving to AISD was not included in the model as >95% of cows had been calved for at least 280 days before treatment started and univariate examination of effects of calving to AISD interval on dependent variables were not significant (P > 0.48). Interval from AISD to conception were compared using analysis of covariance with treatment and BCS entered as factors in the model and age entered as a covariate. Concentrations of progesterone in plasma between cows in the presynchronisation and synchronisation treatment groups were compared using analysis of covariance with the day of detection of oestrus at the first round of AI entered as a covariate.

Submission and conception rates at the third round of AI were not included in the data as numbers were small in both experiments (combined conception rate for both treatments at the third round, Experiment 1: 37.0%, 10/27; Experiment 2: 58.3%, 7/12), both treatment groups were similarly treated when cows were synchronised for the third round of AI, and no specific hypothesis was being tested at the third round of AI. Seven-week pregnancy rates were provided as a guide to the overall reproductive performance of the two treatments during the course of the breeding program.

Oestrous detection rate was defined as the percentage of cows that were detected in oestrus at the presynchronised oestrus or at the first synchronised oestrus. Submission rate was defined as the percentage of cows that were retrospectively classified as not pregnant after the first round of AI that were presented for insemination at the second round of AI (Days 31–35). Conception rate was defined as the proportion of cows that were inseminated that were classified as having become pregnant to that insemination. Pregnancy rate was defined as the proportion of cows that were treated, that were classified as pregnant. This was calculated for periods coinciding with 1, 4 and 7 weeks after the AISD and described as the 1-week, 4-week and 7-week pregnancy rates, respectively. The sensitivity of detection of oestrus was defined as (number detected in oestrus with plasma progesterone <1 ng/mL)/(number with plasma progesterone <1 ng/mL) × 100. Positive predictive value (PPV) for detecting oestrus was defined as (number of correct detections/number of correct + false-positive detections) × 100. Statistical significance for hypothesis tests was set at P = 0.05.

3. Results

Five cows were excluded from analyses relevant to the first round of AI in Experiment 1 because IVDs had not been retained. Another three cows that had lost their IVDs when oestrous cycles were resynchronised for the first round of AI in Experiment 2 were also excluded from analyses.
3.1. Experiment 1

Significant treatment by herd interactions was not detected in Experiment 1 for any of the variables that were analysed ($P > 0.10$).

3.1.1. Reproductive performance

Reproductive performance, oestrous detection rate at the first round of AI and submission rates at the second round of AI for each treatment group in each herd are listed in Table 1. Even though cows in the NI group were not inseminated during the first round of AI, cumulative pregnancy rates after 4 and 7 weeks of mating did not differ significantly between the two treatments (Table 1).

First service conception rates were less in cows that were inseminated at the first synchronised oestrus compared to cows that were first inseminated at the second synchronised oestrus (35.9%, 23/64 versus 66.7%, 42/63, respectively, $P < 0.001$; OR: 0.28, 95% CI: 0.14, 0.58). Artificial insemination start date to conception intervals were not significantly affected by treatment ($P = 0.57$; Table 1), BCS ($P = 0.094$), age ($P = 0.51$) or the interaction of herd with treatment ($P = 0.27$) but cows with shorter calving to AISD intervals had longer AISD to conception intervals ($P = 0.004$).

3.1.2. Concentrations of progesterone and detection of oestrus

Oestrous detection rates at the first round of AI in Experiment 1 averaged 92.0% (127/138) and did not differ significantly between treatment groups (Table 1). The submission rate of cows at the second round of AI was 18% less in the I compared to the NI cows (74.5%, 35/47 versus 92.6%, 63/68; $P = 0.007$, Table 1).

The percentage of cows with low concentrations of progesterone in plasma (<1.0 ng/mL) on Days 34 and 45 during Experiment 1 is listed in Table 2. Insemination at the first synchronised oestrus significantly reduced the percentage of cows with a low concentration of progesterone in plasma on Day 34 on average by 42.8% (range: 28.4–65.0%) but the magnitude of the effect of treatment tended to vary among herds ($P = 0.072$). The percentage of cows with low concentrations of progesterone in plasma on Day 45 did not differ significantly between treatments (Table 2). The percentage of cows in the not inseminated group that had low concentration of progesterone in plasma declined from 96.2% on Day 34 to 5.7% on Day 45. This suggested that most of the cows in that group entered oestrus and ovulated at the resynchronised oestrus.

The sensitivity of detecting cows in oestrus with a low concentration of progesterone in plasma on Day 34 was not significantly affected by treatment and averaged 95.1% (78/82). Values for the positive predictive value of detecting cows in oestrus averaged 98.7% (Table 2). Only one cow was detected in oestrus in Herd C at the second round of AI that had a plasma concentration of progesterone >1.0 ng/mL. No other cows with high concentrations of progesterone in plasma at the second round of AI were detected in oestrus at the second round.

A scatter plot of plasma concentrations of progesterone in cows that were either pregnant following AI at the first round ($n = 20$) or were not pregnant following the first round and were not detected in oestrus at the second round ($n = 13$), is shown in Fig. 2. A total of 38.5% (5/13) and 30.8% (4/13) of the not-pregnant-not-detected-in-oestrus cows, on Days
Table 1
Reproductive performance of cows synchronised for a first insemination (Round 1) and then resynchronised for a second insemination (Round 2) and third insemination (Round 3) that were either inseminated (I) or not inseminated (NI) at the first synchronised oestrus

<table>
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<th>Variable</th>
<th>Herd</th>
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<td>21</td>
<td>24</td>
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<td>70</td>
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<td>Calving to AISD (days)a</td>
<td>42.2 ± 1.4</td>
<td>40.7 ± 1.4</td>
<td>51.5 ± 3.8</td>
<td>45.6 ± 3.4</td>
<td>68.9 ± 5.3</td>
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<td>BCS (1–5)b</td>
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<td>Age (years)</td>
<td>4.4 ± 0.3</td>
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<td>5.1 ± 0.7</td>
<td>6.0 ± 0.6</td>
<td>4.3 ± 0.4</td>
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<td>4.5 ± 0.3</td>
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<td>Oestrous detection rate—Round 1 (%)</td>
<td>95.2 (20/21)</td>
<td>88.2 (15/17)</td>
<td>90.5 (19/21)</td>
<td>91.7 (22/24)</td>
<td>89.3 (25/28)</td>
<td>96.3 (26/27)</td>
<td>91.4 (64/70)</td>
<td>92.6 (63/68)</td>
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<td>1-Week pregnancy rate (%)</td>
<td>47.6 (10/21)</td>
<td>0 (0/17)</td>
<td>23.8 (5/21)</td>
<td>0 (0/24)</td>
<td>28.6 (8/28)</td>
<td>0 (0/27)</td>
<td>32.9 (23/70)</td>
<td>0 (0/68)</td>
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<td>4-Week pregnancy rate (%)</td>
<td>71.4 (15/21)</td>
<td>64.7 (11/17)</td>
<td>52.4 (11/21)</td>
<td>50.0 (12/24)</td>
<td>60.7 (17/28)</td>
<td>74.1 (20/27)</td>
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<td>63.2 (43/68)</td>
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<td>7-Week pregnancy rate (%)</td>
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<td>70.6 (12/17)</td>
<td>77.3 (16/21)</td>
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<td>Submission rate—Round 2 (%)</td>
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<td>88.2 (15/17)</td>
<td>75.0 (12/16)</td>
<td>87.5 (21/24)</td>
<td>90.0 (18/20)</td>
<td>100.0 (27/27)</td>
<td>74.5 (35/47)</td>
<td>92.6 (63/68)</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conception rate—Round 2 (%)</td>
<td>100 (5/5)</td>
<td>73.3 (11/15)</td>
<td>50.0 (6/12)</td>
<td>52.4 (11/21)</td>
<td>50.0 (9/18)</td>
<td>74.1 (20/27)</td>
<td>57.1 (20/35)</td>
<td>66.7 (42/63)</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AISD to conception (days)</td>
<td>9.0 ± 3.6</td>
<td>17.6 ± 4.6</td>
<td>26.6 ± 3.6</td>
<td>22.2 ± 4.5</td>
<td>28.0 ± 3.5</td>
<td>30.6 ± 3.2</td>
<td>21.2 ± 2.0</td>
<td>23.5 ± 2.2</td>
<td>0.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Interval from calving to AI start date.
b Body condition score.
Table 2  
Percentage of cows which were either inseminated (I) or not inseminated (NI) at the first round of artificial insemination in Experiment 1 that were found to have a low concentrations of progesterone in plasma (<1.0 ng/mL) on Days 34 and 45 of the study and the sensitivity and positive predictive value (PPV) of detecting oestrus in these cows at the second round of AI

| Variable | Herd |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|----------|-----|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|          |     | Total    |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Treatment |     |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|          |     | A        | B        | C        |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Low P4 Day 34 |   | 35.0 (7/20) | 100.0 (16/16) | 61.1 (11/18) | 89.5 (17/19) | 65.0 (13/20) | 100.0 (18/18) | 53.4 (31/58) | 96.2 (51/53) | <0.001 |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Low P4 Day 45 |   | 0.0 (0/20)  | 0.0 (0/16)  | 5.6 (1/18)  | 10.5 (2/19)  | 10.0 (2/20)  | 5.6 (1/18)  | 5.2 (3/58)  | 5.7 (3/53)  | 0.62 |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Sensitivity\(^a\) |   | 71.4 (5/7)  | 87.5 (14/16) | 100 (11/11)  | 100 (17/17)  | 100 (13/13)  | 100 (18/18)  | 93.5 (29/31) | 96.1 (49/51) | 0.63 |          |          |          |          |          |          |          |          |          |          |          |          |          |
| PPV\(^b\) |   | 85.7 (5/5)  | 87.5 (14/16) | 100 (11/11)  | 100 (17/17)  | 92.9 (13/14) | 100 (18/18)  | 96.7 (29/30) | 100 (49/49) | 0.38 |          |          |          |          |          |          |          |          |          |          |          |          |          |

\(^a\) Number detected in oestrus with plasma progesterone <1 ng/mL)/(number with plasma progesterone <1 ng/mL) \times 100.

\(^b\) (Number of correct detections/number of correct + false-positive detections) \times 100.
Fig. 2. Scatter plot of concentrations of progesterone in plasma of cows in Experiment 1 that were either classified as pregnant (▲) following the first round of AI or were classified as not-pregnant-not-detected-in-oestrus at the second round of AI (●).

34 and 45, respectively, had plasma concentrations of progesterone <4 ng/mL on these days. For cows that were classified as pregnant following the first round of AI 95% (19/20) and 100% (20/20) had concentrations of progesterone in plasma >4.0 ng/mL on Days 34 and 45, respectively.

3.2. Experiment 2

3.2.1. Reproductive performance

Reproductive performance for cows in the two treatment groups in Experiment 2 are listed in Table 3. Pregnancy rates did not differ between treatment groups at 1 week (\(P = 0.70\)), 4 weeks (\(P = 0.76\)) and 7 weeks (\(P = 0.40\); Table 3) after AISD. Treatment or BCS did not affect (\(P > 0.10\)) any of the dependant variables analysed. Age tended to affect cumulative pregnancy rates after 1 week of mating (\(P = 0.095\); OR: 0.79, 95% CI: 0.60, 1.04), and affected pregnancy rates after 4 weeks (\(P = 0.009\); OR: 0.66, 95% CI: 0.47, 0.92) and 7 weeks of mating (\(P = 0.003\); OR: 0.54, 95% CI: 0.34, 0.85). Odds ratios refer to the changes in the odds of pregnancy for increments of 1 year between 3 and 8 years of age.

3.2.2. Progesterone concentrations and detection of oestrus

The percentage of cows in the presynchrony group that were detected in oestrus at the presynchronised oestrus was 97.7% (Table 3). This was similar to the percentage of these cows that were detected in oestrus following application of the resynchronisation treatment (100%; Table 3). The percentage of cows detected in oestrus at the first round of AI did not
Table 3
Reproductive performance of cows in Experiment 2 that had oestrous cycles presynchronised and then resynchronised for a first insemination or synchronised for a first insemination only

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Presynchronised</th>
<th>Synchronised</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>44</td>
<td>51</td>
<td>95</td>
<td>–</td>
</tr>
<tr>
<td>Calving to AISD (days)</td>
<td>314.2 ± 11.4</td>
<td>306.3 ± 12.2</td>
<td>309.9 ± 8.4</td>
<td>0.64</td>
</tr>
<tr>
<td>BCS (1–5)</td>
<td>2.7 ± 0.03</td>
<td>2.8 ± 0.03</td>
<td>2.74 ± 0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>5.6 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.5 ± 0.16</td>
<td>0.55</td>
</tr>
<tr>
<td>Oestrous detection rate—Presynchrony (%)</td>
<td>97.7 (43/44)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oestrous detection rate—Round 1 (%)</td>
<td>100 (44/44)</td>
<td>98.0 (50/51)</td>
<td>98.9 (94/95)</td>
<td>0.54</td>
</tr>
<tr>
<td>Conception rate—Round 1 (%)</td>
<td>63.6 (28/44)</td>
<td>62.0 (31/50)</td>
<td>62.8 (59/94)</td>
<td>0.87</td>
</tr>
<tr>
<td>1-Week pregnancy rate (%)</td>
<td>63.6 (28/44)</td>
<td>60.8 (31/51)</td>
<td>62.1 (59/95)</td>
<td>0.70</td>
</tr>
<tr>
<td>4-Week pregnancy rate (%)</td>
<td>72.7 (32/44)</td>
<td>76.5 (39/51)</td>
<td>74.7 (71/95)</td>
<td>0.76</td>
</tr>
<tr>
<td>7-Week pregnancy rate (%)</td>
<td>81.8 (36/44)</td>
<td>88.2 (45/51)</td>
<td>85.3 (81/95)</td>
<td>0.40</td>
</tr>
<tr>
<td>AISD to conception (days)</td>
<td>8.21 ± 2.8</td>
<td>10.9 ± 2.8</td>
<td>9.5 ± 2.1</td>
<td>0.49</td>
</tr>
<tr>
<td>Submission rate—Round 2 (%)</td>
<td>68.8 (11/16)</td>
<td>75.0 (15/20)</td>
<td>72.2 (26/36)</td>
<td>0.72</td>
</tr>
<tr>
<td>Conception rate—Round 2 (%)</td>
<td>36.4 (4/11)</td>
<td>53.3 (8/15)</td>
<td>46.2 (12/26)</td>
<td>0.45</td>
</tr>
<tr>
<td>Not-pregnant-not-detected-in-oestrus cows—Round 2 (%)</td>
<td>11.4 (5/44)</td>
<td>9.8 (5/51)</td>
<td>10.5 (10/95)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

differ significantly between the cows in the presynchronisation and synchronisation groups (Table 3).

All of the blood of cows sampled in the presynchronisation (n = 15) and synchronisation treatments (n = 14) had low concentrations of progesterone in plasma on Day 11 (first round of AI) while every cow sampled had concentrations of progesterone >1.0 ng/mL on Day 22. Mean concentrations of progesterone on Day 22 did not differ between the cows in the presynchronisation and synchronisation treatments (8.24 ± 0.57 ng/mL versus 7.13 ± 0.59 ng/mL; P = 0.20). The effect of the day of detection of oestrus at the first round of AI was not significant (P = 0.42). The sensitivity of detecting oestrus in cows that were blood sampled and had low concentrations of progesterone in plasma at the first round of AI in Experiment 2 in both treatments was 100%.

Submission rates at the second round of AI (P = 0.72) and the percentage of cows classified as not-pregnant-not-detected-in-oestrus were not significantly affected by treatment (Table 3). When data from both treatment groups were combined submission rates at the second round of AI were significantly less than the percentage of cows detected in oestrus at the first round of AI (72.2% (26/36) versus 98.9% (94/95), respectively; P < 0.001).

4. Discussion

The results of both experiments support our hypothesis that a reduction in submission rates at a resynchronised oestrus is not due to the treatment that is used to resynchronise oestrous cycles. Rather, it is associated with AI at the first synchronised oestrus. Synchronisation of oestrous cycles without AI followed by resynchronisation of oestrous cycles resulted in over 93% of cows being detected in oestrus. Following AI at either the first induced oestrus (Experiment 1) or at a resynchronised oestrus (Experiment 2) submission
rates of cows at the next oestrus declined by between 10 and 43% in Experiment 1 and 26.7% in Experiment 2. The study design for Experiment 1 involved a comparison of submission rates following resynchronisation of oestrous cycles when cows were either inseminated or not at the first synchronised oestrus. Thus, factors associated with AI were likely to be the cause of the decline in submission rates observed in Experiment 1 following a resynchronised oestrus. In Experiment 2, the absence of a contemporary comparison group without previous AI precludes the same definitive conclusion being drawn. The decline in submission rate recorded at the second round of AI in Experiment 2 could have been associated with AI at the first round of AI or other unknown factors that may have differed between the first and second rounds of AI. In Experiment 2 the recording of similar oestrous detection rates at the first round of AI for the cows that had been presynchronised and resynchronised compared to cows that had oestrous cycles synchronised for a first time supports the second aspect of our hypothesis that a reduction in submission rates following a resynchronised oestrus is not due to use of IVDs and ODB to resynchronise oestrus.

While a direct effect of passing an AI catheter or the extender or cryoprotectant that were used as part of a commercial straw of semen were not tested in this study, it is highly likely that pregnancy and subsequent early embryonic loss was the major factor that caused a reduction in submission rates following resynchronisation of oestrous cycles. Fertilisation rates have been reported to average 88% in heifers and 90% in cows (Sreenan and Diskin, 1986) while calving rates in cows tend to average between 40 and 60% (Morton, 2000a; Royal et al., 2000; Sreenan et al., 2000). Differences between calving rates and fertilization rates suggest that the extent of early embryonic loss approaches 40–50% in cattle. Most of the embryonic loss occurs in the first 2 weeks of gestation, while the rate of loss later in gestation declines (Boyd et al., 1969; Vasconcelos et al., 1997; Dunne et al., 2000). The results of this study suggest that embryonic loss that occurred somewhere between the time of AI and the time of pregnancy testing 13 weeks later reduced submission rates at a resynchronised oestrus by between 10 and 43%. This result is in agreement with the findings of Van Cleeef et al. (1991) who monitored returns to oestrus in heifers that were inseminated and non-pregnant compared to heifers that were not inseminated. In that study heifers were resynchronised from days 17 to 22 after the initial oestrus by treating with an IVD. They reported that only 8.6% (3/35) of the non-inseminated heifers had an extended interoestrous interval while 39% (16/41) of heifers that were previously inseminated, and were classified as non-pregnant, had an extended interoestrous interval of >27 days. The findings of that study also suggested that early embryonic loss was more likely to be the cause of extended interoestrous intervals rather than the resynchronisation treatment that was used.

Failure to detect oestrus has been cited as a cause of reduced submission rates. However, the sensitivity of detecting oestrus in cows that had been resynchronised and which also had a low concentration of progesterone in plasma at the time of the resynchronised oestrus was 95.3% (82/86) in Experiment 1 and 94.1% (16/17) in Experiment 2. These sensitivities of detecting oestrus are similar to the mean sensitivity of 92.5% of detecting oestrus in dairy cows with resynchronised oestrous cycles that has previously been reported (Cavalieri et al., 2003b). The results of both of these studies suggest that while the sensitivity of detection of oestrus plays a role in determining the submission rate of cows at a resynchronised oestrus, factors other than the sensitivity of detection of oestrus may be more important in
influencing the submission rate of cows. Failure to detect oestrus in cows that are classified as not pregnant at the time of a resynchronised oestrus has been shown to be associated with 75% of these cows being in dioestrus at the time of the resynchronised oestrus (Cavalieri et al., 2003b). The results of the present study would suggest that this is due to AI and most likely conception and subsequent pregnancy failure.

Measurement of plasma concentrations of progesterone <4 ng/mL at approximately Day 25 or 35 of gestation could enable some but not all not-pregnant-not-detected-in-oestrus cows to be distinguished from most pregnant cows. Use of, for example, a cow side rapid progesterone test on Day 34 (approximately Day 25 of gestation) could, if the results obtained in Experiment 1 were repeatable across herds, be expected to detect approximately 39% of not-pregnant-not-detected-in-oestrus cows, but the majority of them would be indistinguishable from pregnant cows. Repeated testing, rather than a single test of plasma or milk progesterone, would be needed to differentiate pregnant cows from those non-pregnant cows that have experience an extended duration of the luteal phase of the cycle.

Recent studies have reported that presynchronising oestrus in cows without insemination can increase pregnancy rates when presynchrony is followed by the synchronisation of oestrus cycles commencing on Days 5–12 of the oestrous cycle. Moreira et al. (2001) reported that presynchronisation of dairy cows that were known to be undergoing oestrous cycles with two injections of prostaglandin F2α injected 14 days apart, followed by synchronisation of oestrous cycles with an Ovsynch program, increased pregnancy rates to first service from 34.4% in non-presynchronised cows to 46.9% in pre-synchronised cows. López-Gatius et al. (2003) demonstrated a beneficial effect of presynchrony using PGF2α administered on Days 22 and 36 postpartum, before the start of the mating period, on ovarian activity and early reproductive performance. In Experiment 1, not inseminating cows in Round 1 was associated with an increase in conception rates to first service while presynchrony was not associated with any significant effects on conception rates to first service in Experiment 2. Calving to AISD intervals were relatively short in cows in Experiment 1 and relatively long in cows in Experiment 2. An effect of calving to AISD interval on conception rates to AI has been shown previously with pregnancy rates increasing progressively between 30 and 80 days following calving with little change reported after 80 days (Morton, 2000b; Rhodes et al., 2003). The greater conception rates at the first service in the cows in which insemination was delayed until the second round of AI in Experiment 1 could be explained by the increase in calving to insemination interval that occurred between the first and second rounds of AI, as calving intervals in most cows would have increased within a 30–80 day window. The lack of difference in pregnancy rates at the first round of AI in the presynchronised and synchronised groups of cows in Experiment 2 most likely reflected the fact that the cows enrolled in that study had extended intervals from calving and suggests that there was no benefit of presynchronisation in this population of cows.

In conclusion, the results of this study suggest that AI of cows reduces submission rates of non-pregnant cows following resynchronisation of oestrous cycles after AI. There was no evidence that the resynchronisation treatment was causing a reduction in submission rates at a resynchronised oestrus. Delaying insemination to the second round of AI did not improve 4- or 7-week pregnancy rates in cows. In cows with long calving to AISD intervals, the results of this study do not support a beneficial effect of presynchronisation on conception rates to first service. These results support our hypothesis that a reduction in submission
rates at a resynchronised oestrus is associated with AI at the first synchronised oestrus and not due to a resynchrony treatment involving IVDs and ODB.

Acknowledgments

We thank S. and S. Crooke, J.A. Cirillo and D. Kelly and for the provision of animals for these studies and M. Whitely for conducting hormone assays. Financial support was provided under the Strategic Partnerships with Industry Research and Training Scheme, of the Australian Research Council and the Department of Education, Training and Youth Affairs, and by Genetics Australia and Pharmacia-Upjohn.

References


