Rheinberger Joey (Orcid ID: 0000-0002-2754-7108)
Beggs David (Orcid ID: 0000-0001-5519-9123)

Effect of a second treatment of Prostaglandin $F_{2\alpha}$ during the Ovsynch program on fixed-time artificial insemination conception rates and luteolysis in split calving, pasture-fed dairy cows

JM Rheinberger[*a], DD Colson[b], DS Beggs[a], PD Mansell[a], MA Stevenson[a], RJ Rheinberger[c] and MF Pyman[a]

[a] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia
[b] The Vet Group, Allansford, 260 Ziegler Parade, Allansford, Victoria 3280, Australia
[c] Ironmines Veterinary Clinic, 234 Old Hume Highway, Mittagong NSW 2575, Australia
[*] joeyrheinberger@hotmail.com

Abstract

Objective To compare fixed time artificial insemination (FTAI) conception rates and serum progesterone concentrations at the time of FTAI for cows treated with the original Ovsynch program with those for cows treated with a modified Ovsynch program.

Methods This was a randomised clinical trial in five split-calving, pasture-based dairy herds in Southwest Victoria, Australia. The control group (n = 851) received the original Ovsynch program: day 0 GnRH, day 7 prostaglandin $F_{2\alpha}$, day 9 GnRH and FTAI at day 10. The treatment group (n = 852) received a modified Ovsynch program with an additional prostaglandin injection on day 8. Subsets of cows from each group were sampled for blood progesterone at the time of FTAI.

Results The treatment group had FTAI conception rates that were 7% (95% CI 2%-12%) greater than the control group. After adjusting for the effect of age, days in milk at mating start date and herd, the odds of conception to FTAI was 1.36 (95% CI 1.12-1.66) times greater for treatment group cows compared with control group cows. The variability of serum progesterone concentrations at the time of FTAI was significantly less for treatment group cows compared with control group cows.

Conclusion For Holstein-Friesian and Holstein-Friesian cross-bred cows managed in pasture based dairy herds in southern Australia, the use of a modified Ovsynch protocol including a second injection of PGF on day 8, increased FTAI conception rates compared with cows receiving the original Ovsynch protocol comprised of only a single injection of PGF.

Key words
Ovsynch, dairy cow, prostaglandin, artificial insemination, reproduction, fertility

Abbreviations

AI Artificial insemination
CI Confidence Interval
CL Corpus luteum
DIM Days in milk
FTAI Fixed-time artificial insemination
FSH Follicle stimulating hormone
GCMS Gas chromatography-mass spectroscopy
GnRH Gonadotropin-releasing hormone
LH Luteinising hormone
MO Modified Ovsynch
MSD Mating Start Date
OO Original Ovsynch
PGF Prostaglandin $F_{2\alpha}$ (synthetic analogue)
VWP Voluntary Waiting Period

Introduction

Fixed-time Artificial insemination (FTAI) programs have been developed to synchronise ovulation, allowing cows to be inseminated on a given day, increasing the probability of submission for service once a decision has been made that they are eligible to be bred.[1] The use of hormonal manipulation of the reproductive cycle can improve reproductive performance by decreasing the interval from calving to first service.[2] However, fertility may still be suboptimal when treatments are administered to synchronise inseminations.[3,4]

Ovsynch is an oestrous synchrony program designed to induce ovulation so that FTAI can be used in larger groups of cows.[5] FTAI programs mitigate against the human error that occurs when relying on observing oestrus for insemination and they increase the probability of conception at the time of artificial insemination (AI). The use of FTAI protocols can increase the success of the breeding program, particularly in herds with suboptimal heat detection, through correct timing of insemination. The first treatment in the original Ovsynch program is a gonadotropin-releasing hormone analogue (gonadorelin acetate - GnRH) on day 0. GnRH stimulates the pituitary release of follicle-stimulating hormone (FSH) and luteinising hormone (LH). Following injection of GnRH a surge in FSH precedes emergence of a new follicular wave following which, a follicle is selected to become the dominant follicle and begins to mature. However, the dominant follicle will only proceed to ovulation if its growth coincides with the regression of the corpus luteum (CL). The second treatment in the Ovsynch program is given on day 7 and is intended to cause regression of the CL, allowing a dominant follicle to proceed to ovulation in the absence of progesterone. The third treatment is GnRH on day 9. This causes a surge of LH, which results in ovulation of the dominant follicle. FTAI is timed to occur on day 10, approximately 13 to 16 hours after the second GnRH Injection.[5] Based on recent studies that have assessed progesterone levels for cows undergoing the Ovsynch program, there has been speculation that the hormone injections used are not being administered at the optimum time and/or they should be changed to achieve better pregnancy outcomes.[1]

It has been previously hypothesised that a second injection of PGF on day 8, 24 hours after the day 7 PGF injection of the Ovsynch program would increase the likelihood of complete CL regression and decrease concentrations of progesterone preceding the injection of GnRH on day 9.[6] PGF has a very short half-life and, once absorbed into the blood stream, it is quickly inactivated by oxidation after a single passage through the lungs resulting in a return to pre-injection plasma concentrations by 90 minutes post injection. Thus, a second injection of PGF, given 24 hours later is thought to provide a second opportunity for luteolysis as the probability of luteolysis increases with time following ovulation.[7]

With this background, the aim of this study was to compare FTAI conception rates and serum progesterone concentrations at the time of FTAI for cows treated with the original Ovsynch program with the same measures for cows receiving a modification of the Ovsynch program that involved a second injection of PG on day 8. Increases in FTAI conception rates will decrease Mating Start Date (MSD) to conception intervals. In turn this will improve the synchrony of calving patterns in split and seasonally calving, pasture fed dairy herds allowing the time of calving (and therefore peak lactation) to be timed to coincide with periods of maximum pasture growth.

Materials and methods

This was a randomised clinical trial involving five split-calving, pasture-based herds in Southwest Victoria, Australia. All procedures used in this study were approved by the Animal Ethics Committee of the Faculty of Veterinary and Agricultural Sciences of The University of Melbourne (ethics ID 1513664.1).

Herd selection for this study was purposive and based on agreement of the herd manager to take part in the trial and the presence of an established system for identifying and recording individual cow lactation event information. Herds 1, 2, 4 and 5 were comprised of predominantly Holstein-Friesian cows. Herd 3 was comprised of predominantly Jersey cows.

Conception rates to FTAI using the original Ovsynch program in Australia have been assessed to be in the order of 43%.[8] Based on this information and estimates of the expected improvement in conception rates from a modified Ovsynch program[1,6,9] sample size calculations were carried out to determine the appropriate number of cows to be recruited into the study. We assumed that conception rates for cows in the control group was 43%. If the true conception rate for cows in the modified Ovsynch group was 50%, a total of 796 modified Ovsynch treated cows and 796 original

Ovsynch treated cows (1592 cows in total) were deemed to be sufficient to reject the null hypothesis that the conception rates for treatment and control cows were equal with probability (power) 0.80. The Type I error probability associated with this test of the null hypothesis was 0.05.

The study was conducted from October to November in 2015 and April to May in 2016 to ensure that the program was used across two seasons in a seasonal, split-calving dairying region in Victoria. A total of 1948 cows from five herds were initially recruited into the study at the time of calving. Any cow that did not receive the complete program or had a concurrent disease such as mastitis, metritis or lameness was excluded leaving a total of 1703 cows. In each herd study cows were allocated into two control or treatment groups based on their ear tag number. Cows with even ear tag numbers were allocated to the treatment (modified Ovsynch) group; cows with odd ear tag numbers were allocated to the control (original Ovsynch) group. At MSD for each herd body condition score (using a 1-5 system with 0.25 increments) was assessed for each cow enrolled in the study by the first author[10]. Cows in the modified Ovsynch and original Ovsynch groups were managed as a single herd in each of their respective farms for the duration of the study. Synchrony programs were timed so that the FTAI coincided with the MSD for each herd.

At the start of the study the original Ovsynch group was comprised of 851 cows. All injections given were via the intramuscular route using a 1 ½ inch needle. Each cow in the original Ovsynch group was given an injection of 100 µg GnRH (1 mL GONAbreed®, gonadorelin acetate 100 µg/mL, Parnell Laboratories, Australia) during the morning milking (approximately 0500 to 0800) on day 0, an injection of 0.5 mg PGF (2 mL Cyclase, cloprostenol 250 µg/mL, Boehringer Ingelheim) at the morning milking (approximately 0500 to 0800) on day 7 and a further injection of 100 µg GnRH at the afternoon milking (approximately 1400 to 1600) on day 9 (Figure 1a).

Each cow in the modified Ovsynch group (n=852) received the same treatment program as the original Ovsynch group with the addition of an injection of 0.5 mg PGF at the morning milking (approximately 0500 to 0800) on day 8 (Figure 1b).

Cows in the original Ovsynch and modified Ovsynch groups were submitted for FTAI at the morning milking (approximately 0500 to 0800) 13 to 16 hours after the second GnRH injection. Frozen semen was thawed and insemination carried out by an experienced AI technician. AI technicians for each herd were sourced from one of two established AI companies in consultation with the herd manager. Cows that returned to oestrus following their Ovsynch insemination were submitted for artificial insemination using routine breeding protocols established for each herd.

All cows were submitted for early pregnancy diagnosis between day 35 and 49 after the date of Ovsynch insemination (day 45 and 59 after the start of the Ovsynch program) using a transrectal ultrasound with either a 5 MHz curved linear scanner (Easi-Scan Curve, BCF, Australia) or a 5 MHz sector scanner (5 MHz Duoscan, BCF, Australia). Transrectal palpation was used to confirm pregnancy status for cows that were not detectably pregnant to ultrasound. The timing of this examination meant that only those cows that conceived to the Ovsynch program could be detected as pregnant.

In each herd, subsets of cows were selected at random from both the modified Ovsynch and original Ovsynch groups. A blood sample was taken from each selected cow at the time of first prostaglandin treatment on day 7 and at the time of FTAI.[11] In herds 1, 2 3 and 4 a total of 40 cows were sampled (20 from each group) and in herd 5 50 cows were sample (25 from each group). Blood samples were collected by coccygeal venipuncture into a plain 10 mL vacutainer. Blood collection tubes were placed on ice and centrifuged at 2400 rpm for 4 minutes within 2 hours of collection. Serum (approximately 200 µL) was collected into a separate plain container and stored at -18 °C before analysis. At a commercial laboratory (Gribbles Veterinary Pathology), standardisation of the progesterone test was established using internal standards manufactured analytically and quality assurance samples were run using an established protocol[12]. The sensitivity and assay range was 0.21-60 ng/mL (0.67-191 nmol/L); the minimum detectable progesterone concentration was 0.21 ng/mL and the maximum detectable progesterone concentration 60 ng/mL. Serum concentration of progesterone was determined using a competitive immunoassay using direct chemiluminescent technology (ADVIA Centaur, Siemens Healthcare Diagnostics, USA).

Statistical analyses

Data were entered into a proprietary spreadsheet and analysed using Epi Info 7 (Centers for Disease Control and Prevention, 2014).

Age at calving prior to synchrony, the number of days in milk at MSD, body condition score at MSD and herd were hypothesised to influence FTAI conception rates in addition to Ovsynch treatment group. Age at calving prior to synchrony was expressed as a categorical variable comprised of four levels: 2 years (first calf heifers), 3 to 5 years, >5 years and not recorded. Body condition score at MSD was expressed as an ordinal variable on a 1 to 5 scale. Days in milk at MSD was expressed as a categorical variable comprised of three levels: ≤60 days, >60 days and not recorded. The outcome of interest for this study was the presence or absence of conception following FTAI.

Unconditional associations between each of the hypothesised explanatory variables and the presence or absence of conception following FTAI were computed using the odds ratio. Explanatory variables with unconditional associations significant at the P <0.20 level (2-sided) were selected for multivariable modeling. A fixed-effects logistic regression model was developed where the probability of FTAI conception was parameterised as a function of the explanatory variables with unconditional associations significant at P <0.20, as described above. Explanatory variables that were not statistically significant were removed from the model one at a time, beginning with the least significant, until the estimated regression coefficients for all explanatory variables retained were significant at an alpha level of less than 0.05. Explanatory variables that were excluded at the initial screening stage were tested for inclusion in the final model and were retained if their inclusion changed any of the estimated regression coefficients by more than 20%. Biologically plausible two-way interactions (that is, treatment group × age and treatment group × herd) were assessed and none were found to be significant at an alpha level of 0.05. Herd (a categorical variable comprised of five levels) was included in the model as a fixed effect, regardless of its statistical significance.

Frequency histograms of serum progesterone concentrations for the subset of cows from the original Ovsynch (n = 99) and modified Ovsynch (n = 95) groups that had blood samples taken at the time of first prostaglandin treatment and at the time of FTAI were constructed. The Student's t test was used to test the hypothesis that log transformed serum progesterone concentrations for the two treatment groups differed. The F-test was used to test the hypothesis that the variability of serum progesterone concentrations for the two treatment groups differed.

Results

Descriptive statistics of age, days in milk and BCS at MSD for cows in the original Ovsynch and modified Ovsynch treatment groups are provided in Table 1

Table 2 provides counts of the number of cows that conceived in the modified Ovsynch and original Ovsynch groups and the total number of FTAIs administered to the modified and original Ovsynch group, stratified by herd. The crude incidence risk of conception (referred to as "conception rate" in the remainder of this paper) for the modified Ovsynch group was 49% (95% CI 46% to 52%) compared with 42% (95% CI 39% to 45%) for the original Ovsynch group. FTAI conception rates for cows receiving the original Ovsynch program. Exclusion of cows with existing disease conditions (n = 245 of the 1948 cows that were eligible for enrolment into the study) means that the conception rates reported in this study are likely to be slightly higher than the conception rates achieved when Ovsynch programs are applied to the general population of dairy cows, that is cows with and without disease conditions present at the time of synchrony.

Table 3 lists the estimated regression coefficients and their standard errors from the fixed-effects logistic regression model of factors influencing conception to FTAI conception risk. After adjusting for the effect of age, days in milk at MSD and herd, the odds of conception for cows receiving the modified Ovsynch program was 1.35 (95% CI 1.11-1.64; z = 2.984; P <0.01) times greater than the odds of conception for cows receiving the original Ovsynch program.

While FTAI conception rates varied across each of the five herds included in this study, collinearity between breed and herd meant that it was not possible to quantify the effect of breed on FTAI conception risk. In the herd comprised of predominantly Jerseys (Herd C) conception rates were numerically greater for original Ovsynch cows (45%, 95% CI 34%-56%) compared with modified Ovsynch cows (42%, 95% CI 31%-53%) but the difference in conception rates was not statistically significant at the alpha level of 0.05 (Table 1).

Since previous studies that have assessed conception rates for the modified Ovsynch program have been conducted in herds comprised of Holstein-Friesian cattle only,[1] analyses were repeated after removing Herd C from the data set and in our data, breed was a significant predictor of conception rate. For this subset analysis, after adjusting for the effect of age, days in milk at MSD and herd, the odds of conception for cows receiving the modified Ovsynch program was 1.43 (95% CI 1.16-1.76) times greater than the odds of conception for cows receiving the original Ovsynch program.

Ninety two *percent* of cows in the original Ovsynch group (95% CI 84%-96%) had a serum progesterone concentration of ≥1 ng/mL at the time of first prostaglandin treatment compared with 96% (95% CI 90%-98%) in the modified Ovsynch group (top panel of Figure 2) suggesting a functional corpus luteum that might be responsive to PG[1]. Serum progesterone concentrations (and thus the proportion of cows with functional CLs) at the time of first prostaglandin treatment did not differ for cows in the two treatment groups. Mean serum progesterone concentration for cows in the original Ovsynch group was 21.2 ng/mL; mean serum progesterone concentration for cows in the modified Ovsynch group was 22.2 ng/mL (t test statistic -0.708; df 192; P = 0.48).

Fifty one *percent* of cows in the original Ovsynch group (95% CI 41%-61%) had a serum progesterone concentration ≥1 ng/mL at the time of FTAI compared with 37% (95% CI 28%-47%) of cows in the modified Ovsynch group. Mean concentration of serum progesterone for cows in the original Ovsynch group was 1.9 ng/mL; mean serum progesterone concentration for cows in the modified Ovsynch group of 1.0 ng/mL (t test statistic 3.359; df 190; P <0.01). While both programs were associated with relatively low serum progesterone concentrations at the time of FTAI, the variability of progesterone concentrations for cows in the modified Ovsynch group (standard deviation 0.66 ng/mL) was significantly less than that of cows in the original Ovsynch group (standard deviation 2.77 ng/mL; F statistic 17.54; P <0.01).

Discussion

Fertility is an important determinant of the profitability of seasonally calving, pasture-based dairy herds and, as a result, it is important to identify solutions to factors limiting reproductive performance.[13] In studies undertaken in the USA, average pregnancy rate per AI (a metric comparable to FTAI conception rate) was 38% for lactating dairy cows treated with the original Ovsynch program and subject to FTAI.[5] In Australia, average conception rates of 43% were achieved when lactating cows were treated with the original Ovsynch program and subject to FTAI.[8]

In a meta-analysis Rabiee et al. (2005)[14] found that conception and pregnancy rates with prostaglandin programs (single, double and triple PGF injections 11 and 14 days apart), the Select Synch program (GnRH injection followed 7 days later with a PGF injection and AI to observed oestrus) and variations of the original Ovsynch program (including presynchrony with 1 or 2 PGF injections given 14 days apart with the second injection 12 days prior to the first GnRH injection) were comparable with the original Ovsynch program. While the findings of the Rabiee et al. study show that the Ovsynch program could benefit dairy operations because it allows for timed AI without detection of oestrus, there was little or no improvement in pregnancy rates using Ovsynch compared with programs that relied on the detection of oestrus to determine an appropriate time for AI[14]. A meta-analysis by Borchardt et al. (2017)[15] evaluated the Presynch and (Double) Ovsynch protocols and found that the Double Ovsynch protocol benefited primiparous cows, but not necessarily multiparous cows. The disadvantage of the Double Ovsynch protocol in the split and seasonal calving herds is that it increases the amount of time before AI from 10 days to 27 days. These findings prompted the clinical trial reported in this paper to quantify the benefit (if any) of a modification to the original Ovsynch program to improve FTAI conception rates.

A US study comparing the original Ovsynch program to a modified Ovsynch program similar to that reported in this paper achieved FTAI conception rates of 37% for cows in the modified Ovsynch group compared with 32% for cows in the original Ovsynch group.[9] In another study in the US the addition of a second PGF injection on day 8 of the Ovsynch program resulted in a 38% conception rate for cows receiving the modified Ovsynch program compared with 34% for cows receiving the original Ovsynch program.[1] While conception rates for cows in the modified Ovsynch group were greater than conception rates for cows in the original Ovsynch group it should be noted that conception rates for both groups were substantially lower than the conception rates reported in this study.

In this study the odds of conception for cows receiving the modified Ovsynch program was 1.36 (95% CI 1.12-1.66) times greater than the odds of conception for cows receiving the original Ovsynch program. For seasonally calving, pasture-based dairy herds, increasing conception rates to AI will increase the likelihood of a compact calving pattern in the following milking season and may result in higher rates of genetic gain by increasing the number of animals conceiving to AI prior to the introduction of intact bulls in the later part of the seasonal breeding program. To facilitate comparison with US studies, our analyses were repeated using only those study herds that were predominantly Holstein-Friesian with no appreciable change to our findings. For the Holstein-Friesian herds, the odds of conception to FTAI for cows receiving the modified Ovsynch program was 1.43 (95% CI 1.16-1.76) times the odds of conception of cows on the original Ovsynch program. An additional benefit of improved FTAI conception rates is that if a herd manager uses early pregnancy diagnosis the opportunity exists to subject non-pregnant cows to a second round of oestrous synchrony during the same mating period.

Unlike other studies that report days in milk at the start of the synchrony program as 37 days or similar,[16] there was marked variation in the number of days in milk at the start of the synchrony program (minimum 11 days; maximum 449 days) in this study. This was because cows enrolled in this study were from seasonal- or split- calving systems where the start of the mating period was based on a fixed calendar date (MSD) instead of a fixed number of days post calving (the Voluntary Waiting Period, VWP). Across the five herds, a total of 550 cows of the 1703 enrolled in the study (32%) had been calved 60 days or less at the time of synchrony. While this may not be the optimal time in which to start submitting cows for service, it represents the reality of dairy herd management in Southwest Victoria. In order to account for this, the odds ratio estimates for the modified Ovsynch program reported in Table 2 has been adjusted to account for the effect of days in milk at the time of synchrony start.

In the clinical trial conducted by Wiltbank et al. (2015)[1] assessing the effect of a second PGF injection within the original Ovsynch program in North America, conception rates were compared across parity groups. Wiltbank et al. (2015) found that conception rates for modified Ovsynch cows in their second and third parity cows were greater than conception rates for modified Ovsynch cows in either their first parity or parity four and above. While the findings from Wiltbank et al are broadly consistent with those reported in this study (results not presented) it should be noted that in this study there were substantial numbers of cows with missing data for age (361 of 1703 cows, 21%) making it difficult to provide definitive comparisons of the effect of parity (or age) on responses to the modified Ovsynch program. To avoid loss of statistical power that would have arisen from removal of records from the data set where one or more explanatory variables were missing, we used the indicator method[17] to create a separate 'missing value' category for cows with no value recorded for age. Because this was a randomised clinical trial, the process of randomisation implies that baseline explanatory variables for FTAI conception rate (age, days in milk at MSD and herd) were balanced across treatment groups and therefore not related to the Ovsynch program administered to each cow in the study. Because of randomisation, the distribution of missing values was balanced across treatment groups as well. As a result, both the association between treatment group and FTAI conception rate among cows for whom all data were observed, and the association between treatment group and FTAI conception rate for cows for whom not all data were observed, were reasoned to be unbiased.[17]

Analyses of serum progesterone concentrations showed that, compared with cows in the original Ovsynch group, cows in the modified Ovsynch group had lower serum progesterone concentrations at the time of FTAI and the variability of serum progesterone concentration in this group was less. Our inference from this finding is that a greater proportion of cows in the modified Ovsynch group had complete regression of the corpus luteum, rendering them more likely to be in oestrus (and therefore conceive) at the time of FTAI. The likely reason for a higher proportion of cows experiencing complete luteolysis in the modified Ovsynch group is that the additional dose of PGF provided a second opportunity to induce luteolysis. Since the rate of luteolysis increases with the age of the CL, a second dose of PGF 24 hours after the first would be expected to coincide with the time of responsiveness of the CL to PGF administration of PGF.[7,18] In this study, concentrations of progesterone at the time of FTAI were higher than that recorded in other studies where cows were sampled 48 to 72 h after administration of PGF using GnRH-based treatment protocols.[19] Differences in progesterone concentrations across studies are likely to be due to the use of different assays (e.g. chemiluminescence in this study versus radioimmunoassay in others), differences in the proportion of cows in post-partum anoestrus at the start of treatment programs[20] and differences between beef and dairy cows[19]. Although P4 a cutoff of <1ng/mL has been used in other studies to classify cows as having undergone luteolysis, in our data there appears to be a distinct cut-off point of 3ng/mL at the time of AI in the original Ovsynch group (*Figure 2*) and a cut-off point of 2ng/mL at the time of AI in the modified Ovsynch group (*Figure 2*). This is worthy of further investigation. However, whichever cutoff is used, the proportion of cows that appeared to respond to the PG treatment was higher in the group that received two PG treatments.

Conclusions

For the herds that took part in this study, cows that were treated with a modified Ovsynch protocol that included a second injection of PGF on day 8 had FTAI conception rates that were 7% (95% CI 2%-12%) greater than FTAI conception rates for cows that were treated with the original Ovsynch program, which included only one PGF injection. Economic analyses to determine the cost-benefit of the modified Ovsynch program would be a profitable area of future research.

Acknowledgments

The authors thank The Vet Group, Dairy Australia, Westvic Dairy, Gardiner Foundation, BCF Ultrasound, Boehringer Ingelheim, Gribbles Laboratories, Zoe Vogels and each of the herd managers that took part in this study. This project was also supported through an Australian Government Research Training Program Scholarship.

Conflicts of interest and sources of funding

The authors declare no conflicts of interest. Melbourne University, Gardiner Foundation, Dairy Australia Ltd, Westvic Dairy and BCF Ultrasound provided financial assistance for this work.

References

1. Wiltbank MC, Baez GM, Cochrane F, Barletta R V, Trayford CR, Joseph RT. Effect of a second treatment with prostaglandin F 2α during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. *J Dairy Sci*. 2015:1-11. doi:10.3168/jds.2015-9353

2. Lean IJ, Porter JA, Rabiee AR, et al. Comparison of effects of GnRH and prostaglandin in combination, and prostaglandin on conception rates and time to conception in dairy cows. *Aust Vet J*. 2003;81(8):488-493. doi:10.1111/j.1751-0813.2003.tb13369.x

3. Thatcher WW, Bilby TR, Bartolome JA. Strategies for improving fertility in the modern dairy cow. 2006;65:30-44. doi:10.1016/j.theriogenology.2005.10.004

4. Wiltbank M, Lopez H, Sartori R. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. 2006;65:17-29. doi:10.1016/j.theriogenology.2005.10.003

5. Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL. Pregnancy Rates Per Artificial Insemination for Cows and Heifers Inseminated at a Synchronized Ovulation or Synchronized Estrus. *J Dairy Sci*. 1997;80(2):295-300. doi:http://dx.doi.org/10.3168/jds.S0022-0302(97)75937-X

6. Brusveen DJ, Souza a H, Wiltbank MC. Effects of additional prostaglandin F2alpha and estradiol-17beta during Ovsynch in lactating dairy cows. *J Dairy Sci*. 2009;92(4):1412-1422. doi:10.3168/jds.2008-1289

7. Colazo MG, Martínez MF, Kastelic JP, Mapletoft RJ. Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. *Anim Reprod Sci*. 2002;72(1-2):47-62. doi:10.1016/S0378-4320(02)00087-8

8. Shephard RW. A comparison of performance of the Ovsynch treatment program between cycling and non-cycling cows within seasonally-calving dairy herds. *Aust Vet J*. 2005;83(12):751-757. doi:10.1111/j.1751-0813.2005.tb11588.x

9. Carvalho PD, Fuenzalida MJ, Ricci A, et al. Modifications to Ovsynch improve fertility during resynchronization : Evaluation of presynchronization with gonadotropin-releasing hormone 6 d before initiation of Ovsynch and addition of a second prostaglandin F 2α treatment. *J Dairy Sci*. 2015;98(12):1-12. doi:10.3168/jds.2015-9719

10. Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. A Body Condition Scoring Chart for Holstein Dairy Cows. *J Dairy Sci*. 1989;72(1):68-78. doi:10.3168/jds.S0022-0302(89)79081-0

11. Cavalieri J, Eagles VE, Ryan M, Macmillan KL. Role of the sensitivity of detection of oestrus in the submission rate of cows treated to resynchronise oestrus. *Aust Vet J*. 2003;81(7):416-421. doi:10.1111/j.1751-0813.2003.tb11552.x

12. Siemens Healthcare Diagnostics. Progesterone (PRGE). *ADVIA Centaur ADVIA Centaur XP Syst*. 2011;10629887_E:1-12.

13. Bousquet D, Bouchard E, DuTremblay D. Decreasing fertility in dairy cows: myth or reality? *Med Vet Du Quebec*. 2004;34(JANUARY):59-60. http://www.ivis.org/proceedings/wbc/wbc2004/WBC2004-Bousquet-ang-simple1.pdf.

14. Rabiee AR, Lean IJ, Stevenson MA. Efficacy of Ovsynch program on reproductive performance in dairy cattle: a meta-analysis. *J Dairy Sci*. 2005;88(8):2754-2770. doi:10.3168/jds.S0022-0302(05)72955-6

15. Borchardt S, Haimerl P, Pohl A, Heuwieser W. Evaluation of prostaglandin F 2A versus prostaglandin F 2A plus gonadotropin-releasing hormone as Presynch methods preceding an Ovsynch in lactating dairy cows: A meta-analysis. *J Dairy Sci*. 2017;100(5):4065-4077. doi:10.3168/jds.2016-11956

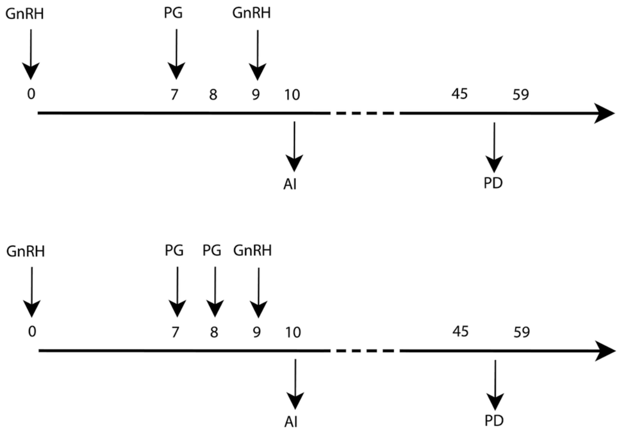
16. Galvao KN, Sa Filho MF, Santos JEP. Reducing the Interval from Presynchronization to Initiation of Timed Artificial Insemination Improves Fertility in Dairy Cows. *J Dairy Sci*. 2007;90:4212-4218. doi:10.3168/jds.2007-0182

17. Groenwold R, White I, Donders R, Carpenter J, Altman D MK. Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis. *Can Med Assoc J*. 2012;184(11):1265-1269. doi:10.1503/cmaj

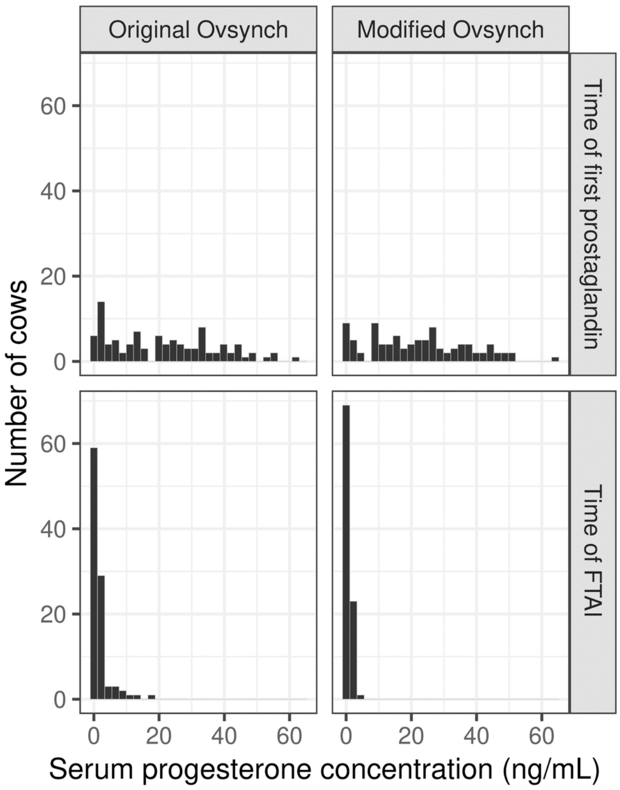
18. Xu Z, Burton L MK. Reproductive performance of lactating dairy cows following estrus synchronization regimens with PGF and progesterone. *Theriogenology*. 1997;47(97):687-701.

19. Stevenson JS LG. Contrasting effects of progesterone on fertility of dairy and beef cows. *J Dairy Sci*. 2016.

20. Stevenson JS, Hill SL, Bridges GA, Larson JE LG. Progesterone status, parity, body condition, and days postpartum before estrus or ovulation synchronization in suckled beef cattle influence artificial insemination pregnancy outcomes. *J Anim Sci*. 2015.



AVJ_12918_AU_Rheinberger_et_al_Ovsynch_Figure-01 (600dpi).tif



AVJ_12918_AU_Rheinberger_et_al_Ovsynch_Figure-02 (600dpi).tif

Table 1: Effect of a second prostaglandin treatment during the Ovsynch program on fixed-time artificial insemination (FTAI) conception rates in split calving, pasture-based dairy herds in Southwest Victoria, Australia. Descriptive statistics of age (in years), days in milk and body condition score at Mating Start Date (MSD).

Variable	**N**	**Mean (SD)**	**Median (Q1, Q3)**	**Min, max**	**Missing**
Age (years):					
Original Ovsynch	851	4.2 (2.4)	4 (2, 5)	1, 19	211
Modified Ovsynch	852	4.4 (3.1)	4 (3, 5)	2, 19	215
Total	1703	4.3 (2.8)	4 (2, 5)	1, 19	426
Days in milk at MSD:					
Original Ovsynch	851	85 (66)	67 (54, 78)	1, 434	180
Modified Ovsynch	852	81 (64)	67 (50, 78)	2, 439	188
Total	1703	84 (65)	67 (51, 78)	1, 439	368
Body condition score					
Original Ovsynch	851	2.70 (0.19)	2.75 (2.50, 2.75)	2.25, 3.25	1
Modified Ovsynch	852	2.70 (0.19)	2.75 (2.50, 2.75)	2.25, 3.50	0
Total	1703	2.70 (0.19)	2.75 (2.50, 2.75)	2.25, 3.50	1

Table 2: Effect of a second prostaglandin treatment during the Ovsynch program on fixed-time artificial insemination (FTAI) conception rates in split calving, pasture-based dairy herds in Southwest Victoria, Australia. Counts of cows receiving FTAI services, counts of cows conceiving to FTAI, conception rate and the odds of conception for treatment (modified Ovsynch) cows compared with control (original Ovsynch) cows.

Herd	Number of cows		CR % (95% CI) [a]	OR (95% CI)
	Conceived (n) [b]	Submitted (n) [c]		
A (Holstein-Friesian):				
Original Ovsynch	72	157	46 (38-54)	Reference
Modified Ovsynch	83	160	52 (44-60)	1.27 (0.82-1.98)
B (Holstein-Friesian):				
Original Ovsynch	57	157	36 (29-44)	Reference
Modified Ovsynch	71	157	45 (37-53)	1.45 (0.92-2.28)
C (Jersey):				
Original Ovsynch	39	87	45 (34-56)	Reference
Modified Ovsynch	34	81	42 (31-53)	0.89 (0.48-1.64)
D (Holstein-Friesian):				
Original Ovsynch	92	208	44 (37-51)	Reference
Modified Ovsynch	120	214	56 (49-63)	1.61 (1.10-2.36)
E (Holstein-Friesian):				
Original Ovsynch	97	242	40 (34-47)	Reference
Modified Ovsynch	110	240	46 (39-52)	1.26 (0.88-1.82)
Total				
Original Ovsynch	357	851	42 (39-45)	Reference
Modified Ovsynch	418	852	49 (46-52)	1.33 (1.10-1.61)

[a] Number of conceptions per 100 FTAI services.
[b] Number of confirmed conceptions to FTAI following the original or modified Ovsynch program.
[c] Number of FTAI services administered following the original or modified Ovsynch program.
FTAI: fixed-time artificial insemination; CR: conception rate to FTAI; OR: odds ratio

Table 3: Effect of a second prostaglandin treatment during the Ovsynch program on fixed-time artificial insemination (FTAI) conception rates in split calving, pasture-based dairy herds in Southwest Victoria, Australia. Estimated regression coefficients and their standard errors from a fixed-effects logistic regression model of factors influencing conception to FTAI conception risk.

Variable	**Conceived** [a]	**Served** [b]	**Coefficient (SE)**	***z***	**P**	**OR (95% CI)**
Intercept	775	1703	-0.1741 (0.1512)	-1.152	0.24	
Treatment:						
Original Ovsynch	357	851	Reference	-	-	1.00
Modified Ovsynch	418	852	0.3093 (0.1008)	3.070	<0.01	1.36 (1.12-1.66) [c]
Age:						
2 years	135	327	-0.3699 (0.1396)	-2.649	<0.01	0.69 (0.52-0.91)
3-5 years	333	670	Reference	-	-	1.00
>5 years	113	345	-0.6000 (0.1439)	-4.105	<0.01	0.55 (0.42-0.73)
Not recorded	194	361	2.9628 (1.1601)	2.554	0.01	19.3 (2.67-397)
Days in milk at MSD:						
≤60 days	173	472	Reference	-	-	1.00
>60 days	407	863	0.3712 (0.1245)	2.980	<0.01	1.45 (1.14-1.85)
Not recorded	195	368	-1.1521 (1.1346)	-1.015	0.30	0.32 (0.02-2.18)
Herd:						
A	155	317	Reference	-	-	1.00
B	128	314	-0.4021 (0.1646)	-2.442	0.01	0.67 (0.48-0.92)
C	73	168	-0.2892 (0.1957)	-1.478	0.14	0.75 (0.51-1.10)
D	212	422	-0.0128 (0.1534)	-0.083	0.93	0.99 (0.73-1.33)
E	207	482	-1.6561 (0.2881)	-5.749	<0.01	0.19 (0.11-0.33)

[a] Number of confirmed conceptions to FTAI.
[b] Number of FTAI services.
[c] Interpretation: After adjusting for the effect of cow age, days in milk at Mating Start Date and herd, the odds of conception for cows that received the modified Ovsynch program was 1.36 (95% CI 1.12-1.66) times of the odds of conception for cows that received the original Ovsynch program.
SE: standard error; OR: odds ratio; MSD: Mating Start Date